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Superstar technology monitored...or throttled?

SUPERSTAR technology is the name given by a working party of the Council for Science and Society to highly innovative, large scale technical projects. A report just issued (*Superstar Technologies*, 66pp, £2.00) looks at "the social mechanisms by which critical expertise can be concentrated on [such technologies], so that grave defects and dangers, technical, environmental and social can be anticipated and minimised". The problem is said to be that there are inadequate mechanisms, particularly at the conception stage of superstar technologies, to monitor possibly disastrous consequences; this is partly because the technology may practically monopolise the employment and commitment of relevant experts, thus weakening the forces of criticism or even commercial competition, and partly because these relevant experts will, being only human, suffer from occasional frailties of intellect, conscience and vision. How, asks the council, can we spot any weaknesses before the project is irresistible?

The answer, with a certain amount of hand-wringing about yet another public institution, is a Technology Implications Commission (TIC) which would "foster the discovery, assessment and diffusion of reliable information about proposed advanced technical projects, so as to provide conditions for effective monitoring, with appropriate public participation at all stages of development". The TIC would neither replace nor interfere with present monitoring facilities further down the line such as select committees or inspectorates. Information would come to it by way of scrutiny of the technical press, patents and so on, "on the lookout for items whose significance might easily be ignored". It would also use independent consultants, and would be a place to which a 'whistle-blower' could turn. The TIC would, if there seemed to be a *prima facie* case for a possible danger, pursue its own independent research and could follow this up, if necessary, either by publicising its findings or holding a conference to generate public debate. The TIC would not have legal power to force the adoption of any policy or the abandonment of any project.

It would be pleasing to be able to give such a modest and mild proposal a wholehearted welcome, firm in the knowledge that it had been hammered out by a representative group after extensive international inquiries. Careful reading of the report suggests otherwise and casts some doubts that the council put together the right sort of mixture. The working party comprised a lawyer, an architect, a professor of physics, a physics research student, a professor of materials science and two science writers. A curious group, markedly, maybe fatally, deficient in practising technologists who might be presumed to know superstar technology from the inside.

It is sad also to relate that the concept of a superstar technology never really comes into focus. Various apparent

candidates put in anecdotal appearances—Flixborough chemical works, the DC-10, Ronan Point (a high-rise block of flats that was severely damaged by a gas explosion), high alumina cement, the Comet, natural gas, supertankers, motor cars, large electrical generating sets, organic chemicals, Browns Ferry, Concorde, boxgirder bridges, thalidomide, Windscale and even groundnuts. All of these are, of course, coupled with disasters, and a mood of disaster informs much of the report. We are told that "the personal safety of thousands . . . and the prosperity of millions may depend upon the engineering success and environmental acceptability of a single enormous project". But the real superstar technologies, on this reckoning, are probably things of the past—railways, electricity and gas distribution, tunnels, sewers, steamships, telephones and transatlantic flight.

The working party claim that changes in the social and financial organisation of industry have weakened some of the forces controlling innovation; Brunel or Edison, for instance, carried heavy personal responsibility for safety and success, which was a continual check on their projects. This is a doubtful argument; Brunel and indeed all Victorian engineers lived with disaster and failure on a scale unknown these days. Society has since then gradually deemed human life less expendable and commercial failure more ignominious. But there has to be a limit to the amount of restraint (even if only moral) on a technologist's freedom of action or else he will give up practising or emigrate. The TIC might come dangerously near to snuffing out what enthusiasm a technologist has.

It is asserted that the control of advanced projects on behalf of society "must depend on the same principle as does science"; this means peer-review and mutual criticism. However well this has served science, it is doubtful whether scientists should go round trying to impose their way of reaching decisions on another community, where decision making is not just a matter of establishing the facts but also of assessing political, social and economic factors. And even 'the facts' are of a different order in technology—the report acknowledges as much when it speaks of the almost impossible task of judging the real weight of innumerable, often contradictory, warnings.

The real danger in all this is, first, that a monitoring agency be generated with which technologists cannot live and, second, that the institutionalising of monitoring may actually create a false public impression that everyone else can relax a little.

There are lots of good intentions behind the council's initiative, and few would quarrel with a call for more open decision making. But the TIC, for all its worthy beaver-ing, might just prove the last straw that broke the not-entirely-wicked camel's back. □



Time for a reappraisal at the Select Committee

THE House of Commons Select Committee on Science and Technology is always an easy target for criticism. It has little money, limited time, few administrative resources. It possesses no legislative power, and shows a frustrating inability to influence intractable government departments.

This bleak picture is made worse by the committee's history of ill-prepared reports and badly formulated proposals, themselves often the product of seemingly sloppy if not uncaring hit-and-miss investigations unbecoming of serious parliamentary procedure. (Remember how the committee championed the ill-fated proposal to appoint a Minister of Research and Development?)

To cap it all, the committee last week published its report on a visit recently made to the Gulf states of Abu Dhabi and Kuwait to investigate "matters of oil policy and related

energy questions". The somewhat inappropriate main recommendation was that more effort should go into the export of Range Rover automobiles to Arab countries. But more disturbing was its remark (an attempt at vindication?) concerning the British-built Steam Generating Heavy Water Reactor (SGHWR): "We were puzzled by reports that the . . . SGHWR cannot be submitted to overseas buyers until a commercial size reactor has been successfully constructed at home."

The committee should be familiar with the problems that have arisen with the Advance Gas Cooled Reactor because of premature scaling-up, and should know that present policy consequently demands stringent, highly detailed design specifications, still not achieved with the SGHWR. The chairman of the committee attempted to justify its thinking with the observa-

tion that Britain should not worry if other states are prepared to accept safety standards less stringent than its own.

The committee's approach suggests that it has become as disillusioned with its own role as everybody else has; an attitude of cynical self-disdain does disservice to British science and technology. The need for well informed, disinterested guidance is perhaps greater now than ever before.

Nobody can be expected to take this committee more seriously than it will take itself. It is time for a reappraisal, a move towards the professionalism and rigour customarily associated with the (admittedly better off) Congressional committees on the other side of the Atlantic. It does not require bottomless coffers and endless time to stop and consider the difference between disinterested and uninterested. □

Seeking sense about scientists in government

The debate over Civil Service scientists in the UK continues. Harold Turner of the National Physical Laboratory gives his view and takes up some of the issues raised in Nature's columns by Cyril Cooper last November and David Budworth in February.

CIVIL SERVICE baiting has become a popular national pastime. The points raised in relation to the science aspects of the sport fall into two main categories: first, those which question the role of the Scientific Civil Service (SCS) within British science and the British economy generally; and, second, those that are concerned with the organisation, structure and competence of the SCS itself. Any evaluation of the criticisms of the SCS must first look at the broader, overwhelmingly more important issues in the former category.

The growth of the SCS arose, not from a doctrinaire belief in Government science, but from the need to provide national scientific services, such as standards and geological surveys, and, in particular, from a desire to compensate for the failure of industry to undertake and apply its own research and development. The beginnings of the decline of investment in British manufacturing industry, and its replacement by the more immediately profitable alternative of invest-

ment abroad, had been perceived by some as early as the 1860s, and the First World War finally brought home to Government that much of British industry was so backward as to bring national survival into question.

The steps thus set in motion—to encourage research in industry, to set up Research Associations and Government research establishments—have led to the pattern we see today. In a very few areas, notably chemicals, industry itself now mounts a research and development effort comparable with that of our major competitors. In a few others, contributing only a small part of our industrial output, there is (for reasons linked with present or past defence policy) an overwhelming concentration of government support. For the rest, representing some 85% of our industrial output, there is an effort which is inadequate by any standards. This maldistribution of effort has persisted for a considerable time, through many successive governments. The failure of our large research and development expenditure (about 2.3%

of GNP) to produce any comparable improvement in economic performance, can be seen to be due to an excessive concentration on high technology, to a general failure on the part of industry to undertake and apply its own research and development, and to a failure of both Government and industry to secure the application in industry of research and development carried out in the public sector and the universities.

In the late 1940s many people, including myself, believed that the technological and competitive weakness of much of British industry could be overcome through a research effort in which Government research establishments played an important part: that the successes of wartime research could be repeated in peacetime conditions. We believed that if we provided research and perhaps some of the development, industry would provide the production and marketing expertise, and investment and economic growth would follow. We thought that our contacts with industry and the involvement of industrialists in the work would ultimately ensure its relevance.

Now, sadly, nearly 30 years later, after passing through phases in which science has been alternately oversold

("the white-hot technological revolution") and undersold (as in the present phase of disillusion), we are wiser and sadder. We know now that the problems of industry are not primarily technological, but economic and managerial, and that we as scientists can have little influence upon them. Many of us now feel that when management and direction in industry is effectual, science will be undertaken or commissioned and applied, but that when management is ineffectual it will not.

The years since 1965 have seen an agonising reappraisal of the role of Government science and particularly of industrial research. There is now probably a reasonable consensus on the rightness of a Government presence in some research and development areas. Thus the provision of certain services, such as standards and specification, would scarcely be questioned. Similarly with work for Government, to provide advice as a basis for statutory or regulatory action (including taxation) and in connection with the procurement function of Government and public authorities generally. Most work for agriculture is welcomed.

But on the crucial question of innovation and growth in British industry there is still violent disagreement on what is the role of Government money and Government science. It is a matter for regret that the Confederation of British Industry (CBI), which could contribute so much, has so far treated these problems at such a superficial level. The SCS seems to have become the national scientific scapegoat. It is dissected and examined as if the solution of all our difficulties is to be found in its entrails. At the back of most official enquiries into the SCS is the feeling that something must be wrong with the organisation and the people if so much expenditure in the end produces so little economic growth. This is not only unjust to the able and dedicated people who constitute the great majority of the staff, but dangerous in that it poses a false and facile solution to a complex national problem.

Take the argument that the SCS is overstaffed and deprives industry and education of much needed scientific manpower. The CBI has campaigned for cuts in the SCS. But it is difficult at this time to take seriously the suggestion that the recruitment of scientists into industry and the schools is limited only or even mainly by the insatiable designs of the SCS. SCS recruitment has been at a low ebb for years, but even so the uncertainty about the future of the service has resulted in a considerable shortfall on advertised vacancies. If industry is not recruiting it is either by intention or

because many young people find industry unattractive, making the issue one on which the CBI might take useful action, for the fault is unlikely to be wholly on the students' side. The problem of science teachers is quite separate: the financial rewards compared with either the SCS or industry must be an important factor.

The suggestion that the SCS is overstaffed is less a valid statement about the staff required for current programmes than an argument for the reduction of the scope of the Service. Sixty per cent of government scientists are employed on defence. Their work is closely related to the Government's procurement function in this area. Staff cuts have already been made and much larger ones seem likely in the next few years. The remaining civil areas have also been cut. Some areas of 'interventionist' work have already been dropped. Attempts to pin down the CBI to say exactly where the line should be drawn between government and industrial research and development have often brought a disappointingly vague response.

Then there is the argument that the SCS as a career service brings seclusion, stagnation, immobility and excessive security. The SCS is a career service because it was created within the Civil Service. The suitability of this form of organisation has often been considered, but the CBI (in evidence to the Fulton Committee) did not advocate the abolition of the career SCS, merely the greater use of short term appointments within it. A proposal to recruit all CS scientists to short term contract posts, with the retention of about 20% after 5 years, came from one individual, Sir Frank Turnbull, a former career administrator at the Department of Education and Science. This proposal was conscientiously considered but rejected by the Fulton Committee. The dangers of stagnation in a career service such as the SCS, though often talked about, have never been investigated "on the ground". There is little to suggest that unproductive scientific civil servants are more prevalent or more of a problem than their counterparts in other walks of life. Where they do exist they are all too often the end product of a chain of management blunders.

This is not to argue that the age structure of the SCS is ideal. However, the crucial factor is not that the numbers have been static for twenty-five years, but that the rapid recruitment in the 1940s and 1950s, falling away in the 1960s and 1970s, has led to an age distribution in which the proportion of older staff is higher than one would choose if there were no constraints. Too many able and effective senior men are inadequately supported. Retirement and movement are already

tending to restore the balance, but in the long run the only satisfactory solution will be consciously to relate recruitment to the long term need for staff at the various levels. Once a long-term view of Government science is evolved this will not be difficult. At the moment, actual interchangeability with industry is still limited and movement out of the SCS is still relatively infrequent. But it may be questioned whether movement does have the unique importance so far attached to it when in some countries, such as Italy and Germany, more successful than we in applying science, it is even less prevalent than here. Movement of scientists in industry to non-research activities is common, but the analogous movement within the Civil Service remains at a very low level despite the recommendations of the Fulton Committee.

Finally, the argument that the SCS is overpaid and provides careers far more favourable than industry. It has been claimed that the typical graduate entrant to the SCS reaches Principal Scientific Officer, and by his late 30s at that. In fact, only around 50% achieve this grade before retirement (a sore point with many SCS graduates), and not all even reach the next lower grade of Senior Scientific Officer. Scientific civil servants are not life-long bench workers; in the defence area particularly, large numbers are occupied in the management of projects funded by government but carried out elsewhere. The role of these members of the Science Group and the nature of their responsibilities has no exact counterpart in industry. It is of course legitimate to compare the SCS with industry, but the chief difficulty in doing so is that while SCS pay and career details are fully accessible to the public, those of industry are not. Information is supplied by industry to the Civil Service Pay Research Unit, but only in the strictest confidence. Comparisons are further complicated by the major differences in the structure of careers between the SCS and scientific employment in industry. In addition there is evidence of a wide scatter of industrial rates. Recent surveys of the remuneration of Chartered members of professional institutions show great differences *within* industry, between different sections of industry, between different locations of employment and between different functions, notably between the scientific worker and the manager of scientific work. An informed discussion of the pay and careers of scientists in all sectors of employment would be welcome, but it ill-becomes the spokesmen of industry to attack the SCS while withholding information on which this discussion alone can be based. □

Rediscovering Islamic Science

An exhibition of Islamic Science opens this week in London—one of many displays, concerts and meetings organised by the Festival of Islam to give a perspective on the Islamic world.

David Davies reports

IT IS very easy to view the historical development of science in terms of the contributions that separate nations or civilisations have made to what we now regard as real, successful, all-encompassing Western science. We get decimals from one place, the lodestone from another, paper from a third and so on. But knowing the history of science only in terms of what we got out of where is like knowing world geography only in terms of what different countries export—a common feature, it used to seem, of school textbooks. In order to understand Islamic science properly, one has to try and identify with Islamic needs and beliefs, and if one is thereby led into alchemy and astrology as well as water-engineering and mathematics, this was no less science in the Islamic world. "We have tried," say Francis Maddison and Anthony Turner in the introduction to their catalogue of the exhibition, "in all modesty, and albeit imperfectly as non-Muslims, to picture Islamic science and technology from within, that is, as part of the intellectual and everyday life of a widely heterogeneous community founded on a common faith."

Wide indeed; the Islam world stretches from Spain to Indonesia, from Samarkand to central Africa. And in time the exhibition, at London's Science Museum, covers nearly a millennium from the first infusion of Hellenic knowledge in the eighth century to the fading away of a specifically Islamic science in the sixteenth and seventeenth centuries. The word science in the exhibition does not, of course, have its present professional connotations of making hypotheses, verifying or falsifying them. It is much more the science-museum/popular view of science as a body of knowledge about things. As far as we know, the doing of experiments in a systematic way was as unknown in the Islam world of the middle ages as it was in the western world of the same period.

Two things cemented the Islam world: the dominance of two main languages, Arabic and Persian, and the importance of religion in everyday life.

The exhibition at the Science Museum is open daily until August 29. Admission 30p; children, students and pensioners 15p.

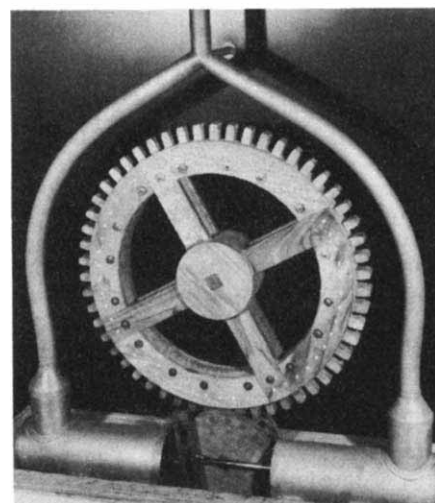
From religious needs sprang much of the science that will be on show. The great eleventh century philosopher al-Biruni commented rather tartly that people were paying more attention to their food, which was a twice-daily concern, than to their prayers, which occurred five times a day. It was in the correct ordering of these prayers that science had a major part to play, both in the accurate measurement of time and in determination of the direction of Mecca so that the prayer mat should be correctly aligned. Thus flourished clocks, sandglasses, astrolabes, astronomical tables, spherical trigonometry and so on. But at the same time there is astrology which was, at a later date in Western science, practised by some but which scandalised others.

The Islamic world stretched east to Indonesia but there is little evidence remaining beyond India of scientific achievements. But Muslims were great travellers, and Egypt and Syria in particular managed at the crossroads to hear about everything.

The exhibition has posed particular problems of what to display. To the scholar the most important sources are the manuscripts, of which there are large numbers. But in planning the exhibition Maddison and Turner have had to keep manuscripts to a minimum simply because they would be generally incomprehensible to a London public. Astrolabes were relatively easy to accumulate for most periods, but the difficulty has been in putting together a representative sample of other objects to exemplify the extent of knowledge. It has turned out that the richest sources of exhibits have been Paris, London and Oxford museums, rather than the Islamic countries themselves. A major disappointment, however, has been that Turkish law forbids even the temporary export of materials from particularly fine collections.

Prosperity in the Islamic world was largely determined by the availability of water, and so the exhibition has had to take note of water technology. But some of the most fascinating examples only existed in manuscript form—notably in al-Jazari's thirteenth century *Book of Knowledge of Ingenious Mechanical Devices*. No one, as far as is known, has constructed any of these devices for seven hundred years, but the recent publication of Donald Hill's

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Modern alchemist's still (above) and (below) the model of a water pump driven by a water wheel

splendid translation (Reidel, 1973) has awakened a wide interest in them. A huge water-clock built specially for the exhibition according to al-Jazari's specifications will undoubtedly be the central attraction for most people. Equally noteworthy, however, will be the half-size model of a water pump driven by a water wheel, also described by al-Jazari. The original was the first recorded pump to be powered other than by human or animal-power.

It is difficult to date the decline of a specifically Islamic science. The growth of the Ottoman Empire in Turkey helped to extend the period, and, both through Turkey and Spain, aspects of Islamic science were siphoned into Western civilisation.

There seems little doubt that the Islamic exhibits being brought together in London, not only of science, but of art, architecture, musical instruments and books, represent a unique occasion. And few believe there will ever in the foreseeable future be another occasion on which so much of the Islamic world can be seen in one city. □

USA

From embargo to control

A committee which has been striving for the past year to draft guidelines to control the use of a revolutionary, but currently embargoed, genetic engineering technique, last week paved the way for many uses of the technique to be resumed in the United States. Colin Norman reports from Washington

FOR nearly two years, many experiments involving the use of a newly-discovered method of manipulating genes in living organisms have been under an embargo because of potential hazards associated with the research. But scientists in the United States who have been itching to use the technique may not have much longer to wait, for it now seems almost certain that the embargo will be lifted in the next few weeks and be replaced with controls designed to minimise the hazards.

The latest step in the tortuous process of developing those controls occurred last week when a committee of the National Institutes of Health (NIH) met to consider criticisms and proposed revisions of a set of detailed guidelines it drafted last December.

The committee stood solidly behind its work, resisting most suggestions that its draft guidelines should be made more restrictive, and it also gave its approval for one particular biological safety system to be used for some of the more hazardous genetic manipulation experiments.

The matter is now firmly in the hands of NIH Director Donald S. Fredrickson, who last week promised to issue a final set of guidelines within a month. Though Fredrickson will probably amend the draft guidelines, a statement he sent to the committee last week indicates that he has accepted the principles embodied in the draft and that any revisions he may make will be relatively minor.

The draft US guidelines seek to contain bacteria or viruses bearing transplanted genes by two methods. First, they spell out four levels of physical containment, ranging from the use of standard microbiological practice to the use of specially designed laboratories (such as biological warfare establishments) capable of handling the most virulent pathogens, and second, they specify that experiments with high potential risk should use viruses or bacteria genetically crippled so as to be virtually incapable of surviving outside the laboratory. The central, and most controversial, portion of the guidelines attempts to assign specific experiments to specific safety levels.

After the committee finally hammered out those guidelines at a meeting last December, Fredrickson sent them out to several scientists for review, and he also called a public meeting to solicit the views of outside groups. The result is a stack of comments more than a foot thick, on the basis of which Fredrickson last week suggested some specific revisions in the draft guidelines and asked the committee for its reactions.

Many of Fredrickson's suggestions simply removed inconsistencies in the draft, and were readily accepted by the committee. But in several instances where Fredrickson proposed changes which would have made the guidelines more restrictive, the committee demurred, arguing that its draft guidelines would provide ample protection against the potential hazards. Specifically, Fredrickson suggested that experiments involving the insertion into bacteria of uncharacterised genes from cold-blooded vertebrates should be placed in the same category as experiments with genes from higher animals (next to the highest level of physical containment, coupled with the use of crippled micro-organisms); he asked the committee to consider the suggestion that genes from other cold-blooded animals and lower eukaryotes should only be transplanted into crippled bacteria; and he also asked the committee for its views on placing all genetic manipulation experiments with the monkey virus SV40 in the highest level of physical containment. Though the committee turned down all those suggestions, it remains to be seen whether Fredrickson will accept its views.

Whatever decisions Fredrickson makes on the fine details of the guidelines, it is clear that many genetic manipulation experiments will require the use of crippled viruses or bacteria before they are allowed to go ahead. In that regard, the committee last week took one important decision and came close to making a bad political blunder on a second.

At its meeting last December, the committee agreed that it should certify whether or not specific strains of viruses or bacteria have been sufficiently crippled to provide the degree of containment spelled out in the guidelines (it took on that assignment largely because no other competent national body is available). At last week's meeting, it was asked to certify two such crippled strains. If it approved them, the effect would be to give the green light to many experiments as

soon as guidelines are published; if it turned them down, the embargo on experiments requiring crippled strains would effectively be maintained because no such strains would be available.

The first strain considered by the committee was a bacteriophage lambda, rendered incapable of surviving at body temperature and carrying three other genetic mutations which limit its infectivity and prevent it from surviving outside an artificial laboratory environment. Developed by Dr Philip Leder and his colleagues at NIH, details of the strain have already been published in *Nature*, and the committee members were provided with test data several weeks before their meeting. They certified the strain as being sufficiently weakened to provide an adequate biological safety barrier.

The second crippled micro-organism under consideration was a strain of the human gut bacterium *E. coli*, developed by Dr Roy Curtiss at the University of Alabama. Curtiss has been working on the strain for more than a year, and he has built a number of ingenious mutations into the organism to reduce its ability to survive outside the laboratory. He has also conducted exhaustive tests to measure the bacterium's chances of surviving. His test data were presented to the committee on the first day of the meeting in the form of a 147-page set of figures, graphs, tables and text. Though few members of the committee had the chance to go through Curtiss's data, they came close to voting on whether or not to approve the strain. Fortunately, however, it was decided—much to Curtiss's relief, it should be noted—to defer a decision for a month to allow committee members to consider the data and confer with other experts.

Though many members of the committee have been keeping in close touch with Curtiss's experiments over the past year, and were therefore well versed in his overall results, if the committee had certified his strain on the basis of such a cursory examination of the data, it would have laid itself wide open to criticism. Biological containment, after all, is a central feature of the safety guidelines. Nevertheless, Curtiss's data is so extensive and by all accounts it provides such convincing evidence for the feebleness of his bacterial strain that the committee is very likely to approve the strain for use in genetic manipulation experiments.

Within the next few weeks, guidelines will thus be issued by NIH to govern that agency's support of genetic manipulation experiments, and the committee's certification of at least one crippled micro-organism has paved the way for a lifting of the embargo in the United States. □

CANADA

Mercury poisoning in Ontario

Are Canada's Indians now victims of Minamata disease? David Spurgeon reports from Ottawa

IN THE spring of 1970, the minister of energy and resources management of the province of Ontario announced that the fish in the English-Wabigoon river system contained high levels of methylmercury and were unfit for human consumption. The sequence of events since then has seen the disruption of the way of life of hundreds of Indians in the area, the closure of a prosperous recreational fishing lodge there, serious doubt cast on the capacity of governments to deal with such issues, and a human tragedy recalling the mercury poisonings at Minamata, Japan.

Dr Masazumi Harada of Kumamoto University in Japan, a neuropsychiatrist and expert on Minamata disease, noted neurological symptoms in many Indians he examined in the area. "There were 37 cases in which some type of sensory disturbance was found," he reported. *"Impairment of the visual field was found in 16 cases. Seven of these cases had asymmetric constriction and tunnel vision. This is very significant in Minamata disease."* (Harada's italics). He was a member of a Japanese delegation that included victims of Minamata disease which last September visited the Canadian settlements affected at White Dog and Grassy Narrows Reserves, near Kenora, in northern Ontario.

The energy and resources minister singled out a paper company's chemical manufacturing plant as the cause of the mercury contamination in the area. Over the previous eight years it was said to have dumped eight tons of methyl mercury into the river system seventy miles upstream.

The process in which the mercury was used has now been replaced, but the advice given by the Ontario government to anglers is to fish for fun only. All commercial fishing licences held by the Indians were revoked in 1970 by the government, which has since supplied them fish to eat from other lakes. This removed a major source of income for the Indians, but it did not stop them eating contaminated fish. And in Grassy Narrows Reserve, where there had never been much reliance on government welfare support, welfare payments are now the principal source of income.

On the government's role, the Canadian Broadcasting Corporation has referred in its programmes to

reports that were ignored or never came to light. Newspapers point to a conflict of jurisdiction between federal and provincial governments.

The line taken by government medical men is revealed by Dr James Stopps, senior consultant on environmental health for the Ontario government, who says "to date no Indian has been diagnosed as having mercury poisoning. That doesn't mean no cases exist; it does mean that after five years we haven't been able to find one." Dr Harada, however, says "even a conservative estimate is that the effect of methylmercury among seven cases cannot be denied." Critics further maintain that the Government's tests were carried out during the mid-winter rather than at the height of the fish-eating season; that it did not test some of the people who ate the most fish (the guides); and that it noted only the mercury levels of the individuals, not their symptoms.

The latest chapter in the story is found in an interim report, published by the federal health department, on a survey of residents of the White Dog and Grassy Narrows Reserves. The purpose of the survey was to determine the extent of exposure to mercury and whether such exposure is causing adverse effects on health; the report which deals with samples of hair and blood obtained at the reserves last August, was written by Dr T. W. Clarkson, professor and director of the Centre in Environmental Health Sciences of the University of Rochester's medical school and a consultant to the Research Institute of the Hospital for Sick Children in Toronto.

"The results obtained to date (December 1975) indicate a wide range of mercury levels—from less than 5 parts per 10^9 to 330 parts per 10^9 in blood," the report said. "The exposure to mercury usually follows a yearly cycle with the highest intake in the summer. Adult males acting as guides at fishing camps have the highest intake of mercury. Five guides out of eleven that were sampled had blood levels in the range of 100 to 330 parts per 10^9 . Analysis of hair samples from the guide having the highest blood concentration (330 parts per 10^9) revealed that the peak mercury concentration had occurred two months prior to the collection of the blood sample. This peak value in hair was close to 150 parts per million, equivalent to a blood concentration of approximately 500 parts per 10^9 ."

The report also revealed that wives

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of guides tended to have higher blood levels of mercury than other females on the reserve. Children usually had lower levels than their parents, but younger children tended to have higher levels than older ones. "A matter of serious concern is the continued exposure of pregnant women to methylmercury," it said. By contrast, analyses of blood samples received from women (mostly non-Indians) living in or around nearby Kenora, and received from the Lake of the Woods Hospital, Kenora, revealed no significant exposure to methylmercury at the time of the delivery of their babies.

A thorough clinical evaluation of the health of those sampled will not appear until phase two of the study. But the report "tentatively" concluded that "several individuals in both reserves still have unacceptably high blood levels of methylmercury . . . very high exposure to methylmercury continues to be a serious problem with guides working at fishing camps." It noted that the highest concentrations reported occurred in the summer of 1975 "despite the fact that warnings against eating contaminated fish have been in effect for several years and that an intensive health education campaign has been in effect during the past year."

The report recommended further action by the guides' employers and health authorities to reduce exposure, and high priority for the planned expansion of a clinical programme. It recommended continuation of the health education programme and—because "the mercury problem may continue for some time"—establishment of a National Centre in Environmental Health. Such a centre, the report said, could deal with other environmental pollutants as well as methylmercury, and "would help detect and deal with potential threats long before they emerged as a national problem." □

SOLAR ENERGY

Power from the sun

Pierre Langereux of La Recherche reports from France following an international symposium on solar electricity held last month in Toulouse

"BEFORE solar energy becomes a major energy source for the 21st century, its development will require financial expenditure of the same order of magnitude as that needed for the development of nuclear energy." So said the scientific director of the National Scientific Research Centre (CNRS), which, together with the National Space Research Centre (CNES), organised the first international colloquium on solar electricity. He was speaking at the end of the meeting, which was attended by about 300 scientists from over 30 countries and produced an agreement on scientific and technical collaboration in developing solar energy through photovoltaic and thermodynamic conversion.

Photovoltaic conversion, perfected 20 years ago by Bell Laboratories and in general use since then to feed man-made satellites, is based on the capacity of certain semi-conductors (silicon, cadmium sulphide, cadmium telluride, gallium arsenide) to transform light energy directly into electricity. Photopiles were developed for use in space exploration, where cost is of comparatively little importance, but they are still much too expensive to be widely used on earth. Silicon photopiles—the only ones currently in commercial use—have been used on earth for about fifteen years already, but for a few limited and highly specialised uses only.

In spite of the promising beginnings, there is still much to be done to make solar energy competitive with the other energy sources already in use. World production of photopiles for terrestrial application is currently equivalent to about 150 KW per year—100 KW in the USA, 30 KW in France, and the rest in Japan and Germany. But the market is expanding. In 1980 the production of silicon photopiles is expected to be of the order of about 10 MW: the price per watt of solar cells (currently \$21) may have been reduced by then to \$5 or \$10 through improvements in the manufacturing process, while in ten years the price could have fallen by a factor of 100 if current research on silicon cells in thin layers bears fruit. The most interesting prospects, however, seem to lie in the development of photopiles using other materials such as cadmium sulphide and cadmium telluride, which were prematurely abandoned for space projects several years ago.

What of thermodynamic conversion? This uses solar ray collectors to heat water which, when transformed into steam, activates an alternator or turbine after passing through a heat exchanger. To reach high power levels, solar towers are needed; heliostats receive solar rays and concentrate them onto a steam generator placed at the top of a concrete tower. The higher the tower, the greater is the capacity of the power station.

Thermodynamic power stations have evolved rapidly, especially in France, and the Sofretes company has recently installed the first solar thermodynamic

power station at La Pas (Mexico). Other projects will follow, with more powerful experimental power stations—one of 1 MW at Odeillo (France), for example, and another of 5 MW in the USA. Both countries also have projects for building 10 MW solar power stations, while in Japan, Mitsubishi Heavy Industries has announced a plan for a 1000 MW solar power station.

Whichever of the two methods is envisaged for the future, however, the most difficult (and perhaps obvious) problem is yet to be resolved: how to store the energy produced by solar power stations, for they can only function when the weather is clear and sunny. In the photovoltaic system, electrochemical storage is expensive; with thermodynamic power stations, on the other hand, one can more easily use thermal storage. Material costs, which have already been studied for nuclear programmes, would be relatively acceptable, and three materials are being considered: oils, rocks and dissolved salts. Great hopes are based also on the possibility of stock-piling hydrogen, obtained as a by-product in the photodissociation of water.

The USA has embarked on a very ambitious programme, with the objective of supplying 5–7% of its energy needs from solar energy in the year 2000, and 25% in the year 2025. The Energy Research and Development Agency (ERDA) will assign \$120 million to the development of solar energy this year, and \$160 million in 1977. France, coming second, has set aside for this year a sum of the same proportionate size relative to the GNP—60–70 million francs, twice as much as in 1975. In comparison, Germany plans to spend only 36 million francs this year, and Japan slightly less. □

INFLUENZA

How it should work

THE House Appropriations Committee has unanimously approved the \$135 million requested by President Ford to inoculate the American public against a possible epidemic of 'swine' influenza next winter. The first batches of vaccine against this new strain should be ready in mid-April for clinical trials of its protective value.

If these are successful vaccination of those most at risk should begin in late summer. Representatives of the drug industry and government health officials testified before Congress this week that they hope to have the whole population inoculated by Christmas.

Rapid production of the vaccine

can be achieved by an ingenious trick devised in the late 1960s. A quick-growing "master" strain of flu virus is hybridised with the swine flu to produce a recombinant bearing key characteristics of the swine flu virus. This recombinant can grow rapidly in fertilised hen's eggs, allowing vaccine production in 6–8 weeks rather than several months. The virus is then collected, killed and packaged into vaccine. It is reckoned that 50 to 100 million hen's eggs will be needed to produce the 200 million shots needed to vaccinate Americans. The protection expected with this vaccine will be between 70 and 80%.

The \$135 million will cover the costs of production and distribution of vaccine only. Vaccination will be voluntary and those who can will have to pay for their flu shots, but

President Ford has stated that nobody will be denied vaccination if he cannot pay for it. The high-risk groups in the population will also automatically receive inoculation against the A and B strains which have been around this winter. But the emergency vaccine itself will only confer protection against the swine flu virus, which on its present showing in the one outbreak so far appears to produce generally less severe illness than the other strains prevalent this winter.

Meanwhile the Canadian government has also decided to approve a mass inoculation programme with swine flu vaccine. It plans to produce about 12 million doses, enough for about half the population, with preference to be given to high-risk groups.

IN BRIEF

Safeguards surface

A few days before taking office as Britain's new Prime Minister, Mr James Callaghan, speaking as Foreign Secretary, revealed in reply to a question in the House of Commons details of the safeguards Britain would apply in deals involving the transfer of sensitive nuclear technology. The safeguards, agreed among the (soon to be enlarged) Group of Seven nuclear exporting countries, demand assurances that the exported technology will not be used to make nuclear explosives for any purpose, that there is sufficient protection against the risk of theft or sabotage and that similar safeguards will apply both to re-export of the technology by the recipient and, for twenty years, to independent replication of nuclear plant by the recipient.

Deadline passed

The United States and the Soviet Union are to continue discussions

which might yield a bilateral treaty on nuclear explosions even though they failed last week to meet the March 31 deadline originally set for agreement. The talks began in July 1974, when Mr Brezhnev and President Nixon agreed in principle that peaceful and military explosions should be limited to 150 kilotons and that there should be some on-site inspection. The remaining difficulty chiefly concerns monitoring multiple peaceful explosions which together exceed 150 kilotons that the USSR might wish to use in civilian projects.

Asbestos inquiry

The renewed controversy in Britain over the health risks of asbestos to the public at large as well as to employees in the industry itself has led to the appointment of a government committee to make a wide-ranging review of the hazards involved and of the safeguards in use. The formation of

the committee, to be headed by the chairman of the Health and Safety Commission, has been welcomed by the industry's Asbestos Information Council, and follows the publication of a report by the Ombudsman criticising the Factory Inspectorate's policing of a plant where 40 people died from asbestosis between 1939 and 1970.

UNEP plea

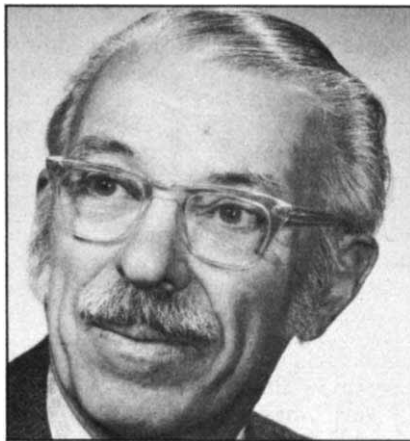
The annual governing council of the United Nations Environment Programme (UNEP), on which 58 states are represented, opened in Nairobi last week with a plea from its executive director, the biologist Dr Mustafa Tolba, that member states fulfil their pledges in order to resolve UNEP's financial crisis and so prevent it curtailing its plans. Only a small proportion of the money pledged has actually been received, with the big countries proving to be the most reluctant contributors.

SPRING in California used to be signalled by a procession of fruit blossoms in the well-kept orchards: almonds in February, then apricots, sweet cherries, peaches, and, finally, the miles of prune trees in the Santa Clara valley that have now been displaced by houses. Most orchards have retreated into the background of the concrete invasion. In a few months, the fruit harvest will start with the sweet cherries, and later the wooden lugs of golden apricots will be loaded for shipment.

As a good Darwinian, I feel sure that the sweet and delectable flavour of apricots, enhanced by plant breeding, is meant to encourage me to pick the fruit and walk away with it. Then I shall devour it, and drop the stony pit on the ground so that the apricot will extend its territory when the seed germinates. But the seed doesn't want to be eaten, for this will destroy the embryo. So, in the seed, there is a substance called amygdalin that decomposes when the seed is macerated. This process liberates an enzyme, beta glycosidase, that hydrolyzes amygdalin to glucose and mandelonitrile, a compound of cyanide and benzaldehyde which breaks down to set free HCN. Death to eaters of apricot seeds! Having summarily disposed of its enemies, the apricot will take over the landscape.

The same game-plan is a hallmark of other members of the rose family, and, indeed, of many other plants—sorghums, lima beans, cassava, clovers and acacias. Almonds and beans have been selected by plant breeders for low content of cyanide.

Cassava is soaked, fermented and leached to remove HCN. Until recently apricot pits were discarded, except occasionally the seeds were crushed and added to preserves to

Apricot retaliation

THOMAS H. JUKES

impart the almond flavour of benzaldehyde. The HCN presumably diffused away during cooking.

A new interest in apricot pits has come about as a result of the "health food" sponsorship of "Laetrile" (amygdalin) for the ineffective treatment of cancer. Dressed up as vitamin B₁₇, which is a completely unjustified soubriquet, Laetrile has become the darling of the diet stores. As a result, the apricot has struck back. Last October, a 34-year-old man residing in San Diego, California, developed symptoms of cya-

nide poisoning after eating apricot kernels. A package of the raw kernels was bought at a health food store and made into milk shakes, following a recipe in an "organic food" publication. The man's wife didn't like the taste, so her husband greedily downed both portions, and, one hour later, "developed forceful vomiting, headache, flushing, heavy perspiration, dizziness and faintness". He was rushed to the emergency room of the local hospital, and rescued by a stiff dose of ipecac. Once again, the process of natural selection was thwarted by human intervention.

The state health department drily states in its report "the minimal number of apricot kernels necessary to cause symptoms or death in humans is not known. Since this and similar products are widely distributed in health food stores for reputed nutritional and medicinal value, the possibility of disease from a large number of kernels should be recognised".

As you can guess, my apparently inhuman sympathies are with the plant world. I treat stinging nettles with respect, and I delight in the blue flowers of larkspur and monkshood. Unlike Bret Harte's "mliss", I do not plan to eat the leaves thereof. I disagree with D. H. Lawrence's idea that the devil himself must have conceived the spines of cacti in a moment of sheer ecstasy. Framed in thorns the exquisite blossoms of the cactus are to look at, not to touch. Plants have every right to tell us to keep our distance. *Hommage à Darwin!*

correspondence

Genetics at the OU

SIR,—The letter by Kacser *et al.* (April 1, page 386) concerning the Joint Universities Genetics Course, raises issues on which formal consultations between the Open University and the Nuffield Foundation are taking place. It contains statements that are false, misleading and prejudicial to Open University students and it is my duty to comment.

Firstly it should be understood that the grant from the foundation is less than half of the costs of production of a half-credit second-level course, the rest being met by the Open University. Nuffield support, whilst generous, was essentially given to carry through a project devised and planned by the participating universities.

We have always had draft material assessed for its academic validity by independent experts before final publication; the genetics course is no exception; all its units have been independently assessed and developmentally tested with volunteer student panels.

Relatively late in the planning of the course, the Nuffield Foundation proposed, and collaborating universities agreed on, a Consultative Committee to aid in course development. The Committee's unanimously agreed rôle was "to advise and comment on the general course outline, aims and objectives" and "to provide a group of geneticists willing to serve as academic referees for particular detailed portions of the course". It had neither control of nor responsibility for the course or the use made of it in individual institutions. In the main this is how the committee has interpreted its rôle and this has been of great assistance to the course team. Inter-university collaboration is a delicate and difficult plant to nurture, and it is a pity that the harmony between different universities reflected in the work of the team has not carried through to all the members of the consultative committee.

Kacser *et al.* state that "the Committee informed the Foundation that it was dissatisfied with the outcome of the previous three years' work and asked that the course should not be published, or even used by Open University students, without further consideration, restructuring and amendment". This is untrue. A docu-

ment signed by some members of the Committee (whose membership is incorrectly given in the letter), which was neither seen nor discussed at any meeting of the committee, and whose very existence was unknown to some of its members, was passed to the Director of the Nuffield Foundation.

Kacser *et al.* imply that the committee thought that the length and depth of the course was far beyond the capacities of the intended students. They state (as if they had only just discovered the fact) that for Open University students the study time for the average "Unit" is 12 hours, and that this work must be completed, on average, in a week. All this is, of course, perfectly true. But they then go on to assert that "it was the Committee's considered opinion" (though the Committee has never considered such an opinion) "that this is an impossible task if understanding at a University level is aimed at".

This workload is normal for all Open University courses. If the opinion of Kacser *et al.* is correct, the fact that many thousand students have successfully performed this "impossible task" over the past five years could only mean that these students did not achieve "understanding at a University level". This is a gratuitous insult to students and graduates and a grave reflection on our academic staff and external examiners.

Further, as Kacser *et al.* know full well, developmental testing of the course's components has continued this year with actual students. To pronounce upon the alleged difficulty and overloading of the course in advance of the completion of this evaluation seems to indicate prejudice rather than a spirit of objective criticism. To do so publicly seems to me to be irresponsible. Based on the first returns we have had from students, the average times taken to study Units 1 and 2 were 7 hours and 10 hours respectively, 99% of students reported on Unit 1 as being "OK" to "Very Interesting" and 98% found it "OK" to "Very Easy" to understand. The comparable figures for Unit 2 were 78% in each case. It would be premature to generalise from the first two units, but the facts so far contradict Kacser *et al.*'s mere opinion. I am confident that when the evaluation of the remaining Units—and the examiners' assessment of students' performance—has been completed, Kacser

et al. will be shown to have been wrong.

Kacser *et al.* further suggest that the use of this course by other institutions "has not been seriously considered". Quite the contrary: the other participating universities have, I believe, already made plans to use sections of it in their own teaching programmes and several other institutions have already let us know of their plans to do the same. It is also misleading to claim that an "obvious deficiency" of the course is that it has no "practical manual". At an early stage the team discussed practical work and decided that because of the widely differing circumstances of the different institutions that might use the course, no attempt should be made at present to prepare a manual except for OU students.

Kacser *et al.* assert that "the Consultative Committee jointly with the Nuffield Foundation suggested a number of changes which would have gone some way to meet these criticisms, but they were not accepted by Professor Rose". This is false in one respect and misleading in another. At no time has the "Consultative Committee jointly with the Nuffield Foundation" suggested these changes. Of course, in the design and development of the course, comments from members of the committee have been submitted to the team; many of these were accepted, with appreciation. The statement misleadingly suggests that acceptance or otherwise of comments was the prerogative of Professor Rose. In fact, all such decisions were taken by the full course team.

I very much regret the inaccuracy and tone of the letter, and wish to reiterate my belief that this course is academically and educationally on a par with other second-level Open University science courses, the value and success of which is already well established. Because of the wide interest in the subject and the way in which the course is constructed, the course will be of use in many other teaching contexts than the Open University.

Yours faithfully,

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news and views

Ectopic production of HCG subunits

from a Correspondent

THE synthesis of a particular hormone or hormone subunit by tumours derived from a tissue not normally engaged in its production (ectopic production) is now a familiar observation. Endocrine abnormalities in patients can often be traced to the presence of a tumour in non-endocrine tissue. Measurement of raised levels of some hormones circulating in the blood is often used as a biochemical marker of malignancy and to monitor the success of surgery, analogous for example to the use of chorionic gonadotropin assays in women with choriocarcinoma. Human chorionic gonadotropin (choriogonadotropin) (HCG) is synthesised by the trophoblast at a very early stage of pregnancy—within a few days of fertilisation. It provides a critical support to the corpus luteum, which decays in its absence with resulting menstruation, and so is crucial in establishing and maintaining pregnancy. HCG is not normally present in the circulation of non-pregnant females, the only exceptions being patients with choriocarcinoma (Vaitukaitis *et al.*, *Ann. J. Obstet. Gynecol.*, **113**, 751–758; 1972), hydatidiform mole (Crawford, *Brit. Med. J.*, **4**, 715–719; 1972) and patients with cancers that ectopically synthesise the hormone (Braunstein *et al.*, *J. clin. Endocrinol. Metab.*, **35**, 857–862; 1972; Braunstein *et al.*, *Ann. intern. Med.*, **78**, 39–45; 1973). HCG can also be detected in normal testis (Braunstein *et al.*, *New Engl. J. Med.*, **293**, 1339; 1975).

The ectopic production of chorionic gonadotropin in cell lines was first described in Gha-Go cells isolated from

a bronchogenic carcinoma (Rabson *et al.*, *J. natn. Cancer Inst.*, **50**, 669–674; 1973; Tashjian *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1419–1422; 1973). Both subunits of HCG were identified. The α subunit of HCG (HCG- α) is nearly identical to the α subunits of thyroid-stimulating hormone (thyrotropin), follicle-stimulating hormone (folli-tropin) and luteinising hormone (lutro-pin) whereas the β subunit (HCG- β) is different from the β subunits of the other glycoprotein hormones. The α and β subunits are glycoproteins containing moderate amounts (10–20%) of carbohydrate. Two papers in *Nature* have now shown that these subunits are synthesised and secreted by HeLa cells in culture.

Ghosh and Cox (*Nature*, **259**, 416–417; 1976) used the HeLa 65 and HeLa 71 sublines. They showed by radio-immunoassay with β subunit antiserum that the secretion of small but significant amounts (~ 0.0024 ng per 10^6 cells over 5 d) of immunologically reactive material occurs during culture. It is not clear from the results of Ghosh and Cox whether β subunit production, secretion of the complete hormone or both processes takes place. Furthermore, there is a crucial question of the degree of specificity of antiserum for β subunit, as considered later. Perhaps the most striking result reported by Ghosh and Cox is the greatly increased (1,000-fold or more) secretion of immunologically reactive material when HeLa cells were grown in medium supplemented with 1–5 mM butyrate. The response of the cells to butyrate varied almost linearly up to 20 ng per 10^6 cells over 5 d with butyrate concentration over the range 0–5 mM. This intriguing finding is apparently not specific to HCG production. The levels of activity of placental alkaline phosphatase, which like HCG is produced ectopically by HeLa cells (Elson and Cox, *Biochem. Genet.*, **3**, 549–561; 1969) are also markedly increased by butyrate (Griffin *et al.*, *Arch. Biochem. Biophys.*, **164**, 619–623; 1974). The mechanism of action of butyrate in augmenting synthesis of two glycoproteins is unknown. It may be significant that short chain fatty acids including butyrate, were shown to increase the activity of a sialyl transferase in HeLa cells (Fishman *et al.*,

Biochim. biophys. Acta, **59**, 292–299; 1974), since sialylation of at least one glycoprotein, thyroglobulin, seems to be obligatory for its secretion (Monaco and Robbins, *J. biol. Chem.*, **248**, 2328–2336; 1973). The substrate specificity of the HeLa cell sialyl transferase(s) responding to butyrate may therefore be of interest in considering its possible role in controlling HCG secretion.

Although the Ghosh and Cox experiments do not distinguish between secretion of β subunit or completed HCG molecules, Lieblisch *et al.* (this issue of *Nature*, page 530) show unequivocally that at least some sublines of HeLa secrete relatively massive quantities of α subunit ectopically (about 75 ng per 10^6 cells per day). Unfortunately the HeLa sublines (CCL 2, 2.1 and 2.2) used by Lieblisch *et al.*, do not include those studied by Ghosh and Cox. Since the rate of secretion of α subunit varies about 50-fold among the various sublines studied by Lieblisch *et al.*, it is possible that HeLa 65 in fact secretes only the β subunit. Alternatively these cells may carry out a balanced synthesis of α and β subunits with secretion of the completed hormone.

The relatively massive secretion of free α subunit by the HeLa sublines studied by Lieblisch *et al.*, does however raise the question of what Ghosh and Cox were measuring in their basal system or even in the stimulated cultures. The antiserum used by the latter workers, raised against purified β subunit, is stated to show less than 1% cross reactivity with luteinising hormone indicating even less than this degree of cross reactivity with the common subunit. But such contamination could conceivably account for the small reactivity obtained in the medium of cells grown in the absence of butyrate. It is of course less likely to complicate the measurement of the much larger quantities of immunologically reactive material produced by HeLa 65 cells in butyrate medium although this cannot be entirely ruled out.

The α subunit secreted in such large amounts by HeLa cells is immunologically indistinguishable from α subunit isolated from pregnancy urine. The tumour α subunit is apparently not structurally identical however,



A hundred years ago

ON the 31st ult. a meeting, at which several well-known English biologists were present, took place at the house of Dr. Burdon-Sanderson, at which the advisability of establishing a society or association for the purpose of promoting the progress of physiological research in England, was considered and discussed.
from *Nature*, 13, April 6. 455; 1876.

since Liebllich *et al.*, found a small but definite difference in elution of the tumour α subunit during gel filtration compared with the pregnancy subunit. A slightly increased molecular weight could account for this discrepancy, although glycoproteins often behave abnormally during gel filtration chromatography. Weintraub *et al.* (*J. clin. Invest.*, **56**, 1043–1052; 1975) showed similar abnormal properties for α subunits secreted by the Gha-Go cell line. In gel chromatography the Gha-Go subunit behaved as a protein of 27,000–30,000 apparent molecular weight, significantly higher than the α subunit found in pregnancy (23,000–24,000 apparent molecular weight). Thus,

there seems to be an increase in the size of α subunit made by tumours. It may be that this material represents a precursor form present only transiently in the production of normal α subunit and HCG by placental cells. Interestingly, the Gha-Go tumour α subunit could not form biologically active HCG when incubated with HCG β subunit and tested in a rat testis radioreceptor assay. By contrast HCG α subunit from pregnancy urine combined efficiently with HCG β subunit to reconstitute 95% or more of the expected HCG activity under the same conditions. It seems therefore that tumour α subunits may be defective in a stage leading to functional HCG molecules. The mole-

cular basis for the difference is presently unknown. It is possible that the α subunits are degraded in some way by tumour-specific proteases, although this seems unlikely from their apparently greater molecular size compared with the subunit of pregnancy urine. Their amino acid compositions are also very similar to pregnancy α subunit except for small differences in some residues, particularly valine, phenylalanine and half cystine. Perhaps further analysis by peptide mapping or comparison of carbohydrate contents might suggest the structural differences between biologically active α subunit and the apparently inactive product of tumour cells. $\square$

As a general rule, animals settle disputes with other members of the species by means of ritualised displays rather than by physical violence. In contests for food, territories and mates, raised crests and songs serve as substitutes for teeth and claws, and the loser retreats unscathed. This much is well known, but it is less clear why the ritualised communication system does not break down because of cheating. It is easy to imagine that the winner of a ritualised fight could save itself the risk of future harassment by killing the loser, yet even animals with powerful jaws or horns rarely do this; instead they play by the rules. Similarly, if a dominant individual claims a territory by, for example, loud song, why do submissive individuals not bluff their way to success by singing?

The first attempt at a formal theoretical analysis of these problems was made a few years ago by John Maynard Smith (*Nature*, **246**, 15; 1973; *On Evolution*, Oliver and Boyd, 1972). Using game theory, Maynard Smith was able to analyse why physical violence (which he calls escalated fighting) does not replace the 'conventional' system of ritualised signals. His method of analysis was to look for an evolutionarily stable strategy, or ESS. An ESS is defined as a strategy which, when it is used by the majority of individuals in a population cannot be bettered by any alternative rare 'mutant' strategy. The 'strategies' which Maynard Smith considered included such alternatives as 'hawk'—an individual which always escalates—and 'mouse', which only plays the conventional game and retreats at the first sign of violence. He then assigned payoffs for winning, losing, getting injured in an escalated fight and so on, and used these values to construct a payoff matrix for each strategy against all others, including itself. It is intuitively clear that the

hawk strategy does very well against a mouse, but does poorly in a population made up mainly of other hawks, because the risk of injury is high, so it is not an ESS. Only in the extreme case where losing a contest means certain death, say from starvation, will hawk persist as the best strategy. Otherwise the most likely ESS, for a wide variety of payoff values, was a mixed strategy of mainly ritualised display, but with escalation in retaliation to violence by an opponent. Fortunately, this seems to be the

Why don't animals cheat?

from John Krebs

common pattern of contest in real animals. In another model Maynard Smith (*J. theor. Biol.*, **47**, 209; 1974) showed that if a species has no method of escalating (because it does not possess dangerous weapons) so that the main cost involved in a contest is time, the ESS is again a mixed one with the distribution of persistence times given by the expression $(1/v) \exp(-x/v)$ where v is the value of winning and x the persistence time.

In a recent paper, Maynard Smith and Parker (*Anim. Behav.*, **24**, 159; 1976) have taken the analysis a step further by considering combats in which there is an asymmetry. In the real world two contestants will rarely be identical; one may be larger than the other, or may have more to lose, say because it has invested effort beforehand in establishing a territory.

In the latter case, where the payoffs are unequal, it is an ESS for the individual with more to lose (the territory holder for example) to invest more in the contest. Although there is a 'paradoxical' ESS in which the intruder invests more, Maynard Smith and Parker show that this ESS

is unlikely to occur in nature, because, unlike the common sense ESS, the 'paradoxical' strategy cannot invade a hypothetical ancestral population which ignores the asymmetry. In the other type of asymmetry, where differences such as size give an indication of the probable outcome of an escalated fight, the ESS depends on how accurately each individual assesses its own size in relation to that of the opponent, and how good size is as an indicator of the outcome of a fight. It turns out that size, or some other such cue, will be used as a conventional means of settling a contest if the contestants estimate it with certainty, even if it is a poor predictor of the outcome of a fight. The ESS will be "always retreat if you think your opponent is larger, always escalate if you think he is smaller, and do something intermediate if he is the same size".

This automatically leads one to consider the question of bluffers. If it is an ESS for animals to use features such as size to settle asymmetric contests, can bluffers, individuals with the equivalent of padded shoulders, spread through the population? Maynard Smith and Parker conclude that bluff will always tend to spread but it is probably limited by the ability of other individuals to detect the difference between bluff and real fighting ability.

At the moment, this new approach to analysing ritualised contests is still in the theoretical stage, but some data seem to support the theory. Male dung flies (*Scatophaga stercoraria*) competing for females at newly deposited cowpats show the negative exponential distribution of persistence times predicted by the 'no escalation' model. Although this in itself is not a very surprising result, the fact that males with different persistence times have an equal chance of mating suggests that the distribution is in fact an ESS.

Monocular deprivation and the input to the visual cortex

from A. M. Sillito

THE role of visual experience in establishing the functional properties of the developing mammalian visual system is a topic which has aroused considerable controversy and has been the subject of a recent review in *Nature* (Barlow, *Nature*, **258**, 199; 1975). There is however general agreement in this field that a normal input from the two eyes in early life is essential for maintaining an effective binocular input to visual cortical neurones. If monocular deprivation is produced in a kitten by suturing together the eye-lids of one eye at eye opening time for a period of two months or more it will be found that the majority of cortical neurones will be driven only by the non-deprived eye instead of both eyes as in a normal animal (Wiesel and Hubel, *J. Neurophysiol.*, **26**, 1003; 1963). There is a critical period during which monocular deprivation is effective in producing these changes. In kittens it extends from some time in the third week of life to approximately three months and the effects of deprivation extending through the critical period seem to be irreversible despite subsequent long periods of normal binocular vision (Hubel and Wiesel, *J. Physiol.*, **206**, 419; 1970). Even quite brief periods of monocular deprivation during the first month of the critical period will result in the deprived eye's appearing to lose access to cortical cells and an essentially complete takeover by the non-deprived eye (Peck and Blakemore, *Expl Brain Res.*, **22**, 57; 1975). However, reverse suturing (opening deprived eye, suturing the normal) within the critical period can result in the previously deprived eye's regaining its ability to drive cortical cells and a complete reversal of the original situation can even occur in which all the cells are driven by the previously deprived eye (Blakemore and Van Sluyters, *J. Physiol.*, **237**, 195; 1974).

Of particular interest at present are the mechanisms contributing to the effects of monocular deprivation. Although there are morphological changes in the layers of the lateral geniculate body (the thalamic relay between the eye and visual cortex) receiving an input from the deprived eye the functional properties of these cells seem to be relatively unchanged (Wiesel and Hubel, *J. Neurophysiol.*, **26**, 987; 1963). In fact binocular deprivation produces similar morphological changes in the lateral geniculate body but does not result in a dramatic loss of binocularly-driven neurones (Hubel and Wiesel, *J. Neurophysiol.*, **28**, 1029;

1965). This type of evidence together with the results of reverse suturing experiments carried out within the critical period have led to the view that there is a competitive interaction at the cortical level between the afferent terminals deriving from the respective eye inputs. It has been suggested that there may be an actual growth and reorganisation of afferent terminals (Movshon and Blakemore, *Nature*, **251**, 504; 1974) with the implication of a loss on cortical cells of some or all the terminals deriving from the deprived eye. Alternatively, it can be argued that during the critical period synapses that are used become 'more effective', whilst those that are not are in some way 'silenced' although still present, and that within the critical period this is a reversible process. A recent paper (Duffy, Snodgrass, Burchfiel and Conway, *Nature*, **260**, 256; 1976) has presented some important new evidence on this topic. They found, in cats monocularly deprived by lid suture from the fourth week of life to eight months, that intravenous injection of bicuculline revealed an input from the deprived eye to cells that were otherwise only driven by the normal eye. Thus they managed to reverse briefly the effects of monocular deprivation and furthermore they found that within this period, the receptive field properties in the two eyes were basically the same. The alkaloid bicuculline is an antagonist of γ -aminobutyric acid (GABA) in the central nervous system and GABA is a putative inhibitory transmitter in the retina, lateral geniculate body and visual cortex. Bicuculline applied iontophoretically to visual cortical neurones will produce changes in their receptive field properties that are consistent with a loss of an intracortical inhibitory input (Sillito, *J. Physiol.*, **250**, 305; 1975). Hence the results of Duffy *et al.* suggest that the deprived eye actually retains an input to cortical cells but that this is in some way suppressed by GABA-mediated inhibitory process. It is tempting to view this simply as a block of intracortical inhibition but the presence of GABA-mediated inhibition at other levels in the visual system means that intravenously injected bicuculline will produce changes in excitability throughout the system and this may contribute to the observed effect.

Whilst no one would dispute that monocular deprivation from the fourth week of life to the eighth month produces an irreversible loss of the ability to drive cortical cells from the deprived eye, it must be noted that in the experi-

ments of Duffy *et al.* the deprivation started in the fourth week and not at eye-opening time. This means that the animals had approximately one week of binocular visual experience partially overlapping the beginning of the critical period. This is reflected by the similarity of the receptive fields in the deprived eye to those in the normal, an occurrence which if deprivation had been from eye-opening time might not be expected in so far as the receptive fields would be more likely to be of the immature type (Blakemore and Van Sluyters, *J. Physiol.*, **237**, 195; 1975). It is important that the effects of intravenously injected bicuculline should be examined in a situation where monocular deprivation is started at eye-opening time, and hence where there will have been no opportunity for initial reinforcement of binocular input to cortical cells. Probably the most crucial experiment would be to examine the effects of iontophoretically applied bicuculline on visual cortical cells in monocularly deprived cats to see whether a local block of GABA-mediated intracortical inhibition can reveal an input from the deprived eye. With the present evidence in mind there seems little doubt that some type of inhibitory suppression of the input of the deprived eye, either with or without a concurrent loss of synapses or synaptic effectiveness, constitutes an important component of the cortical effect of monocular deprivation. □

T antigen and DNA synthesis

from Walter F. Mangel

POLYOMA and SV40 virus induce an intranuclear tumour (T) antigen early in lytic infection in transformed cells and in tumour-bearing animals. T antigen appears in lytically infected cells at the same time as the early virus-specific 19S mRNA and is followed by the induction of chromatin replication. This temporal sequence of events led to the hypothesis that T antigen is an early virus-coded protein which is responsible for the induction of cellular DNA synthesis that occurs after infection by papova viruses. In three recent articles from the laboratory of A. Graessmann in Berlin, direct experimental support consistent with this hypothesis is presented.

If T antigen is the early gene product

of polyoma, then microinjection of early polyoma RNA sequences into the cytoplasm of permissive cells might result in a positive immunofluorescence test for T antigen (Graessman *et al.*, *Nature*, **258**, 756; 1975). So polyoma DNA was symmetrically transcribed by the *Escherichia coli* DNA-dependent RNA polymerase. This complementary RNA (cRNA) contained both early and late viral sequences because the early virus-specific cytoplasmic RNA originates from 50% of the E strand of polyoma DNA and the late mRNA from 50% of the L strand (Kamen *et al.*, *Cold Spring Harb. Symp. quant. Biol.*, **39**, 187; 1974). On removing the DNA, the biological activity of the cRNA (1 mg ml⁻¹) was assayed after microinjection of about 10⁻¹¹ ml into individual secondary mouse tissue culture cells. Intranuclear T antigen was first detectable 6 h after cRNA microinjection in 6% of the cells. Thereafter the number of T antigen-positive cells as well as the intensity of T antigen-specific fluorescence per cell increased, reaching a maximum of 70% of the cells between 20 and 24 h, before declining to undetectable levels 65 h after cRNA microinjection. V antigen (capsid protein) which is a late virus-coded protein (Mangel, Hewick and Smith, unpublished) appeared with the same kinetics as T antigen.

Microinjection of asymmetrically transcribed SV40 cRNA which contains the early mRNA sequences not only results in T antigen formation but also in the induction of cellular DNA synthesis (Graessmann and Graessmann, *Proc. natn. Acad. Sci. U.S.A.*, **73**, 366; 1976). Between 24 and 26 h after microinjection, 70–80% of the epithelial cells of confluent primary mouse kidney cultures were T antigen positive. In pulse labelling experiments, the maximum proportion of cells synthesising DNA was reached between 15 and 20 h. During this time interval, 85% of the T antigen-positive cells were incorporating tritiated thymidine whereas only 3.2% of the mock injected cells in control experiments were synthesising DNA.

Important controls for these experiments included the demonstration that the biological activity of the cRNA was destroyed by RNase but not DNase. Although actinomycin D inhibited T antigen formation in mouse cells either infected with wild type polyoma virus or microinjected with polyoma DNA, it did not reduce the cRNA-induced T antigen formation within the first 15 h. Inhibition of protein synthesis by cycloheximide, added to the cells after cRNA injection, prevented T antigen formation. Finally, neither total RNA nor polyadenylated RNA isolated from uninfected cells induced T antigen

synthesis on microinjection.

In a series of preliminary experiments to confirm that the early genes induce T antigen the four fragments of SV40 DNA produced by the two restriction endonucleases *HpaI* and *HpaII* were individually microinjected into monkey cells (Graessmann *et al.*, *FEBS Lett.*, **61**, 81; 1976). Only one fragment induced T antigen synthesis and none induced V antigen synthesis. The fragment, which corresponds to 36% of the total genome, contains only 60% of the early region and thus could code for a protein with a molecular weight of up to 60,000. This is the size of the largest polypeptide immunoprecipitable by T antibody that is synthesised in the wheat germ cell-free system using asymmetrically transcribed SV40 cRNA as message (Roberts *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1922; 1975). But with early SV40 mRNA as message in the wheat germ system, the largest immunoprecipitable polypeptide synthesised has a molecular weight of about 90,000, which implies that the entire early region codes for T antigen (Smith *et al.*, and Prives *et al.*, in *In Vitro Transcription and Translation of Viral Genomes* (edit. by Haenni, A., and Beaud, G.), **47**, 331 and 305, Editions INSERM, Paris, 1975). The results of Tegtmeyer (*Cold Spring Harb. Symp. quant. Biol.*, **39**, 9; 1974) indicate that the *in vivo* T antigen has a molecular weight of about 90,000. Thus the polypeptide induced by the *HpaI* and *HpaII* fragment may only contain some antigenic sites for T antibody and be biologically inactive. It would be interesting to know whether this fragment can induce DNA synthesis and transform cells.

The microinjection experiments support the hypothesis that T antigen formation requires early viral RNA sequences which must be translated and that all cells synthesising T antigen within the first 25 h of SV40 cRNA microinjection are also stimulated to synthesise DNA. The results are consistent with T antigen being the early virus-coded protein. They do not, however, exclude the possibility that T antigen is a cellular protein which is antigenically modified by the cRNA translation product. To show that T antigen is entirely a virus-coded protein, asymmetrically-transcribed SV40 cRNA must be translated *in vitro* and the product's tryptic peptides proved to be identical with those from T antigen isolated from transformed cells. Once T antigen is purified, the microinjection technique may be used to determine whether this protein is both necessary and sufficient for the induction of DNA synthesis as well as for transformation, an experiment whose results are eagerly awaited. □

Microtextures and rock deformation

from K. R. McClay

The rheology of rock masses in the Earth's crust and upper mantle has been the object of intensive study over the past decade, encompassing phenomena from the formation of slaty cleavage and mylonite genesis, to peridotite deformation. Results of research on these topics from schools in France, Germany, the Netherlands, Belgium, Great Britain, Australia, and America were presented at the recent conference on Fabrics, Microtextures, and Microtectonics, held on March 3–5, 1976, at Leiden, the Netherlands. The conference was organised by the Department of Tectonics, Leiden University, and papers arising will be published in a special issue of *Tectonophysics* later this year.

THE aim of the conference was an assessment of the role of fabric, microtextural and microtectonic information in the interpretation of geological processes. Microtextures (often called microstructures) are the morphological characteristics of rocks, embracing features from microfolding and cleavages to dislocation substructures. The recognition of a particular microtexture as being characteristic of a certain deformational and thermal environment, is fundamental in structural geology. Relict microtextures, overprinting relationships and microtextural transitions are all used in the estimation of temperature, pressure and rate of deformation for a geological event.

Fabrics are the distribution in space of discontinuities in rock masses, (that is, preferred crystallographic orientations). Increased application of X-ray texture goniometry and of metallurgical theories has generated a new impetus in fabric studies. Model studies of fabric development indicate that it may be possible to use fabrics as an aid in the determination of the deformation environment, strain history and the kinematics of deformation.

For a considerable time, the study of fabrics and microtextures was essentially the collection of data with little interpretation. It now seems possible that a number of fabrics and microtextures may be understood in terms of the kinematics of deformation—particularly the evolution of foliations, mylonites, and sheared peridotites.

The first part of the conference dealt

with materials amenable to metallurgical techniques, and it became clear that research emphasis has changed from finite strain studies to kinematics, with the realisation that the kinematics of the deformation history have an important role (for example B. Hobbs (Monash University, Australia), and A. Nicholas (University of Nantes, France)). Crystallographic preferred orientations may reflect the influence of deformation paths as clearly documented in the field studies of quartz mylonites by J.-L. Bouchez (University of Nantes) and by J. Carreras, A. Estrada (University of Barcelona), and S. White (Imperial College London). It was shown that the quartz *c*-axis fabric is dependent upon the deformation path of the mylonite. The experiments on marble by E. Rutter and M. Rusbridge (Imperial College) support the theoretical predictions of the influence of the deformation path on the development of preferred orientations—particularly the predictions (G. Lister, Leiden University) that the new preferred orientations overprint the old fabric patterns after approximately 30% strain.

Theoretical fabric analysis using a metallurgical approach is a most exciting development. Using a Taylor-Bishop-Hill model, Lister has computed *c*-axis preferred orientations for quartz polycrystals utilising various combinations of slip systems. This allows simulations of deformations at a number of metamorphic grades to be made and the computed fabrics agree well with those found in naturally deformed quartzites. A. Etchecopar (Montpellier University) has used a different approach to fabric modelling with a two-dimensional single slip analysis which gives agreement with specific quartz and peridotite shear fabrics. These models allow postulates about the deformation mechanisms and the strain history of the rock to be made. For the first time a clearer understanding of fabric development and its complexities is at hand. In conjunction with fabric models, G. Davies (University of Cambridge) analysed the preferred orientation development in a deformed metal and demonstrated that, with the aid of orientation distribution functions, it is possible to assess the relative roles of various deformation elements such as glide, twinning and phase transformations during fabric development. Orientation distribution analysis, coupled with theoretical modelling is a potentially powerful tool in understanding rock fabrics.

Dealing with recrystallisation processes during or after deformation of a metal, J. Jones (McGill University, Canada) pointed out that the differentiation between dynamic recovery, cyclic, continuous, or annealing

recrystallisation was of fundamental importance in the development of fabrics and microtextures. A study of the deformation behaviour of single crystals of galena at various strain rates and temperatures (K. McClay and B. Atkinson, Imperial College) indicated that dynamic recovery and recrystallisation processes in galena occur at surprisingly low temperatures (for example migration of kink band boundaries at 200 °C).

Other important concepts brought out included the role of the yield surface both in single crystals and in polycrystals in determining the response of the material to an applied stress or to an imposed strain. In addition, Burgers vector and glide plane determinations seem critical in allowing further advance in theoretical models for deformation and fabric development. S. White (Imperial College) pointed out that subgrains may have a memory of deformation conditions and discussed in detail the problems associated with the use of dislocation substructures in rocks in which a substantial amount of recovery has occurred during or after deformation. This difficulty was also discussed in the light of an extremely interesting report on thermal decoration of dislocations in peridotite nodules (Y. Gueguen, University of Nantes).

In the second part of the conference, contributions on the formation of foliations in highly anisotropic minerals were discussed. P. Williams (Leiden University) reviewed foliation development, emphasising the role of initial kinking of phyllosilicate in producing foliations and indicating that the foliation will reflect the XY plane of the finite strain ellipsoid during all stages of development only in special cases. Experimental contributions to the study of foliated rocks since 1960 were reviewed by W. Means (State University of New York). His experiments using salt/mica analogues produced very good approximations to natural crenulation cleavages and foliations as defined by mica films in deformed sandstones.

An extremely interesting contribution in this section on highly anisotropic minerals was that of A. Maltman (University College of Wales, Aberystwyth). His experiments on soft sediment deformation produced structures simulating those found in zones of incipient slaty cleavage formation. R. Knipe and S. White (Imperial College) reported on a detailed high voltage electron microscopic study of slates showing the change from a crenulated fabric in the low strain zones around a fold to a well developed slaty cleavage at higher strains. Excellent high magnification micrographs give us

our first good look at the fine structures which can be resolved optically only with great difficulty. Evidence for the growth of new phyllosilicates during the crenulation process was clear. A study of glacier deformation by M. Hambrey (Geological Institute, ETH, Zurich) illustrated the complexities of foliation development.

It can be concluded that the increasing application of metallurgical techniques and theories in a kinematic approach to rock rheology and microtextural problems is a promising direction for research. In particular, certain quartz fabrics can be explained using a kinematic approach. There are also important changes in the interpretation of foliations and cleavages. Initial crenulation of an already existing bedding plane fabric seems to have an important role in cleavage generation. The relationship between the cleavage plane and the finite strain ellipsoid may not be important. In all, the emphasis has changed from finite strain analysis to studies of deformation mechanisms and kinematics in order to understand both the strain paths and deformational processes in geology. □

Mobile spaghetti

from P. V. E. McClintock

ONE of the central assumptions of Vinen's 19-year-old theory of the supercritical state of superfluid ⁴He has been challenged by Ashton and Northby in a recent issue of *Physical Review Letters* (35, 1714; 1975) on the basis of their experiments at the University of Rhode Island.

The superfluid properties which liquid ⁴He displays below its transition temperature at 2.18 K can only be maintained if the liquid is treated fairly gently. Large fluxes of heat or high velocities of flow can cause the superfluidity to disappear abruptly, taking the liquid into a so-called supercritical state. The nature of the supercritical state which results when a large thermal current is passed through the liquid formed the subject of Vinen's historic series of papers in the *Proceedings of the Royal Society* (A240, 114 and 128, 1957; A242, 493; 1957; 243, 400; 1958), and it is this topic which has again been under study, using a different experimental technique, at Rhode Island.

Below its transition temperature liquid ⁴He behaves as though it were an intimate mixture of two interpenetrating fluids: a superfluid component, with zero viscosity, able to indulge in non-dissipative flow at low

velocities; and a normal fluid component which has ordinary liquid properties and carries all the thermal energy. For small thermal fluxes, the liquid is almost a superconductor of heat, its being virtually impossible to set up a significant temperature gradient. This is because of a very efficient internal convection mechanism, whereby there is a flow of superfluid towards the heater, where it is converted into normal fluid and then flows away again through the oncoming superfluid, carrying heat towards the cold end of the apparatus. There is thus a counterflow of the two components without any net movement of the liquid. As the heat input is increased, however, a critical level is eventually reached at which the liquid undergoes a sudden transition to the supercritical state, and a finite temperature gradient spontaneously appears.

On the basis of a series of elegant experiments, Vinen was able to show that this discontinuous change in behaviour was a consequence of the appearance in the liquid of a tangled mass of vortex lines. Just as in any other fluid, a vortex in helium consists of a narrow linear core region about which the fluid (for ^4He , only the superfluid component) rotates with a velocity inversely proportional to its radial distance from the centre: an interesting difference, however, is that the circulation, or "strength", of superfluid vortices in helium, being quantised, is always the same, which introduces enormous simplifications into any calculations of the supercritical state. According to Vinen, when the velocity of counterflow of the normal and superfluid components exceeds a certain critical value, vortex lines are generated and, by providing a dissipative mechanism, they enable a temperature gradient to be set up. In his model the vortex lines form an irregular tangle, with their cores being arranged somewhat like a bundle of spaghetti, but in continuous motion because each vortex exists in the flow patterns of all its neighbours and, indeed, of other parts of itself. It is assumed that vortex line is created at constant rate by the normal-superfluid counterflow, but that there also exists a mechanism by which the lines are destroyed: when two elements of line with opposite directions of circulation approach closely enough they can annihilate each other. Competition between the creation and annihilation mechanisms therefore results in an equilibrium length L_0 of vortex line per unit volume characterising the vorticity. Vinen proposed that, in the equilibrium state, the tangle of lines was both homogenous and isotropic. The latter proposition also implied that the vorticity would, on average, be at

rest relative to the superfluid so that, in a thermal counterflow experiment it would be moving towards the heater. What Ashton and Northby have now accomplished is the first satisfactory measurement of the direction and the velocity with which the equilibrium tangle of vortex lines moves relative to a laboratory frame of reference.

Their experimental apparatus, constructed from the insulating material Lucite, was in essence exceedingly simple. A heater was placed against the inside end wall of a 10 cm long channel of rectangular (0.5 cm $\times$ 1 cm) cross section, whose other end was left open to the helium bath in which the cell was immersed: by sufficiently energising the heater, the helium in the channel could thus be brought into the supercritical state, with a thermal counterflow of the normal and superfluid components occurring along the channel between heater and bath. At right angles to this counterflow, recessed in the side of the channel, was an arrangement for generating ions and directing them into the liquid, where some became attached to the vortex cores (there being an attractive force between an ion and a vortex arising from the kinetic energy of the rotating superfluid which the ion replaces). Any given ion, after being held like this for a while, was subsequently released as a result of the annihilation of the element of vortex line on which it had been trapped. An electric field was provided to draw freed ions to a pair of electrodes—one nearer the heater, the other nearer the bath—mounted on the opposite side of the channel from the ion generator. By noting whether trapped ions were being released upstream or downstream from the ion generator, it was possible to determine unambiguously in which direction the tangle of vortex lines was moving, and it was also possible to measure its drift velocity.

In the event, Ashton and Northby discovered that, quite contrary to the picture proposed by Vinen, the vorticity does not move with the superfluid component; furthermore, they have been able to show that it actually moves in the opposite direction, that is, in the same direction as the normal fluid component. The existence of a net relative velocity between superfluid and vortex lines implies that, to some extent at least, the vorticity is anisotropic.

These new results do not seem to be in conflict with other aspects of Vinen's model, but his theory is almost certainly going to need some significant modifications. The authors suggest a possible approach, but further experiments are clearly required before one can be sure they are right. $\square$

No surprises for vitamin B₁₂

by Peter Newmark

It is always a bitter sweet experience when a highly sophisticated investigation does little more than confirm what lesser studies had already more or less demonstrated. The pleasure that is gained from confirming the predicted plot is countered by the disappointment at finding no unexpected twists, let alone a surprise ending. A good illustration of that principle is to be found in a recent study of transcobalamin II (TCII), the plasma protein that transports the cobalamins (vitamin B₁₂) between tissues (Schneider *et al.*, *J. clin. Invest.*, **57**, 27; 1976).

For some years it has been known that most of the vitamin B₁₂ in human blood is not attached to TCII but to the completely distinct transcobalamin I (TCI), one of a family of vitamin B₁₂-binding glycoproteins. Vitamin B₁₂ attached to TCI is only slowly transferred into the tissues. If the vitamin is bound to TCII, however, it is rapidly cleared from the blood. This knowledge has been obtained with the aid of radioactive cobalt-labelled vitamin B₁₂ and it has therefore been impossible to determine directly the fate of the transcobalamins, although tissue uptake of TCII has been indicated.

In 1972 Allen and Majerus succeeded in applying affinity chromatography to the isolation of the transcobalamins. As a consequence Allen and his colleagues have since been able to prepare radioactive iodine-labelled transcobalamins and to study their fate simultaneously with that of the labelled vitamin bound to them. When human TCI-vitamin B₁₂ was injected intravenously into rabbits, clearance of both protein and vitamin was at about the same slow rate as that of labelled bovine serum albumin (Burger *et al.*, *J. biol. Chem.*, **250**, 7700 and 7707; 1975) unless the TCI was desialylated, when both protein and vitamin were extremely rapidly cleared into the liver in a manner reminiscent of that described by Morell *et al.* (*J. biol. Chem.*, **246**, 1461; 1971) for various desialylated glycoproteins.

A different picture emerged when similar experiments were repeated with TCII-vitamin B₁₂ (Schneider *et al.*, *J. clin. Invest.*, **57**, 27; 1976). In the first place the native protein and its vitamin disappeared rapidly from circulation and second, their destination was widespread rather than being confined to the liver.

Since, initially at least, the transcobalamin moiety of the complexes always accompanied the vitamin, the

information gleaned by having the protein independently labelled was not much more than had been known from studies of crude transcobalamins with labelled vitamin alone. A poor return, one might think, for having to produce, for labelling, transcobalamins purified several million-fold from several hundred litres of plasma.

More original, although also predicted, was the data obtained on the fate of the TCII following its uptake into tissues. After a 30-min time lag radioactive iodine appeared in the urine in rapidly increasing amounts and with a molecular weight of less than 1,000, indicating linkage to, at most, a small peptide. That can only mean that TCII had been rapidly degraded in the tissues. In contrast the vitamin was either retained in the tissues or returned to the blood stream, with little appearing in the urine.

Whereas it is easy to understand why the vitamin should be retained it is, as Schneider *et al.* point out, harder to explain the wasteful way in which TCII is destroyed in the process (compare transferrin). Various microorganisms appear to be able to release vitamin B₁₂ from TCII and it is difficult to see why cell surface enzymes could not have evolved to do the same.

Tissue uptake of transcobalamin-vitamin B₁₂ complexes probably involves pinocytosis, a process that threatens to come back into vogue in relation to receptor modulation (Raff, *Nature*, **259**, 265; 1976). Regardless of that there is little doubt that the transcobalamins are destined to receive more attention in the near future. In part that is thanks to the possibilities opened up by Allen's elegant techniques and in part it is motivated by despair. This year is the 50th anniversary of the discovery by Minot and Murphy (for which they were to share a Nobel prize with Whipple) that a diet of raw liver could control the previously fatal pernicious anaemia. But we are still at a loss to understand the biochemical basis for the prevention of that disease by vitamin B₁₂, the active component of liver. □

Central anomalies: why so strong?

from Peter J. Smith

WHEN marine magnetic anomalies were first analysed in detail in the early 1960s, it immediately became clear that their amplitudes decrease with distance from the corresponding oceanic ridge. Possible explanations for this phenomenon were not long in coming. For example, the magnetic constituents of the oceanic lithosphere newly formed

along ridge axes may change chemically with time, giving rise to a gradual decrease of magnetisation. Another suggestion was that older igneous crust would be overlain by a thicker deposit of sediment; so the anomalies as measured at the ocean surface would be attenuated with respect to those observed above younger crust with less accumulated sediment.

But although these and some other processes, acting either individually or together, could possibly explain a gradual decrease in anomaly amplitude, they were apparently insufficient (at least in the form originally envisaged) to account for the precise form of decrease actually observed. For there is usually a particularly large reduction in amplitude immediately beyond the central anomaly followed by a much more gradual decrease outwards towards the continental margins. In the case of slow-spreading ridges (<30 mm yr⁻¹ half-rate), for example, the central anomaly amplitude can be at least twice as high as those of near neighbours, although for faster-spreading ridges the difference is less marked.

More recently, near-bottom magnetic measurements have shown that not only does the central anomaly have a relatively high amplitude, there is often also a large-amplitude, short-wavelength (<15 km) anomaly within it. With slow-spreading ridges the latter is not generally observed separately at the water surface because of loss of resolution with distance from the ocean floor; the wider and narrower anomalies combine in such cases to give a very large central anomaly. Over fast-spreading ridges, on the other hand, the two anomalies can usually be clearly distinguished from each other.

One possible explanation of the relative strength of the wider central anomaly, first proposed by Matthews and Bath (*Geophys. J.*, **13**, 349; 1967), is that all magnetised oceanic crustal blocks except the central one are contaminated with material of opposing polarity, thereby reducing the strength of their respective anomalies. Harrison (*J. geophys. Res.*, **73**, 2137; 1968), on the other hand, concluded that this phenomenon would be insufficient, suggesting that there must also be a 'demagnetising effect' outside the central region. Klitgord (*Earth planet. Sci. Lett.*, **29**, 201; 1976) has now taken up the question of the demagnetising effect in the particular context of the narrower central anomaly. From a new analysis of both surface and near-bottom magnetic data from many different spreading ridges, she concludes that the narrower anomaly is due to a zone of high magnetisation coinciding with the region of most recent extrusion. The absolute strength of this

magnetisation is regarded as that to be expected from newly-formed basalt recently quenched in seawater. Its large magnitude relative to that of the adjacent older basalt, however, is attributed to the fact that the latter rock has undergone low-temperature oxidation.

This is not the first time that oxidation has been seen as the cause of decreasing anomaly amplitudes. Klitgord's point, however, is that in the light of recent work oxidation can be invoked to explain not only the gradual reduction of anomaly amplitude away from a ridge but also the more sudden drop at the edge of the central anomaly. The recent work concerned is partly that of Butler (*J. geophys. Res.*, **78**, 6868; 1973) who concluded that small stable single-domain titanomagnetite grains become superparamagnetic when oxidised to titanomaghemite and thus lead to a greater reduction in magnetisation than the oxidation of pseudo-single-domain or multi-domain grains, and partly that of Watkins *et al.* (*Earth planet. Sci. Lett.*, **8**, 322; 1972) who found that fresh pillow basalt has a zone of fine grains immediately below the glassy outer rim.

The argument is that many of the fine grains in this zone will be single domains with high magnetisation, which means that they will contain a high percentage of a pillow's total magnetisation. A given amount of alteration in the zone will therefore have a much greater effect on the overall magnetisation than the same amount of alteration in the larger-grained pseudo-single-domain interior of the pillow. Moreover, being both fine-grained and close to the exterior, the zone will be much more susceptible than the interior to alteration in the first place. Rapid oxidation of the fine-grained zone is thus envisaged as the cause of the sudden amplitude drop outside the narrower central anomaly, whereas slower oxidation of the interior is seen as the cause of the more gradual amplitude decrease away from the ridge.

The widths of the narrow anomalies in the ridge systems studied by Klitgord range from 2 km to 10 km, which compare with several tens of kilometres for the main central anomalies. The magnetic relationship between the two anomalies never clearly emerges, however. At one stage Klitgord suggests that "the narrow magnetisation high probably does not account completely for the difference in amplitude between the central anomaly and the flanking anomalies", implying that it accounts for some, and perhaps even a high (but unspecified) proportion, of the difference. On the other hand, it is acknowledged that in some cases the narrow anomaly does not exist at all.

articles

Effects of drugs on the processes regulating the functional activity of brain 5-hydroxytryptamine

A. Richard Green & David G. Grahame-Smith*

5-Hydroxytryptamine (5-HT), seems to be involved in depression and perhaps schizophrenia. A behavioural test of 5-HT action in animals has been used to clarify the mechanism of its action and the effects of various drugs used clinically in affective disorders.

5-HYDROXYTRYPTAMINE (5-HT) is present in the cell bodies and varicosities of neurones in specific tracts in the brain^{1,2}. Much is bound to synaptic vesicles³ and there is good evidence that it is synthesised at the sites at which it is found. In view of the circumstantial evidence, and in the absence of evidence to the contrary, it is assumed here that it acts as an orthodox neurotransmitter (see ref. 4). It has been suggested that 5-HT is involved in the hypothalamic control of body temperature⁵, the sleep-waking cycle⁶, pain sensation⁷, and sexual behaviour⁴. There has been discussion about its role in schizophrenia⁸, affective disorders⁴, and morphine addiction⁴.

To understand the physiological role of 5-HT and the effects of drugs on those systems in which it is involved, it is essential to consider the regulation of 5-HT synthesis and release and how both of these are concerned in its functional activity. There are probably four factors involved in presynaptic regulation: synthesis, compartmentation, intraneuronal metabolism and reuptake. These four processes are presumably integrated to control the release of appropriate amounts of 5-HT from the presynaptic nerve terminal, as well as the amount acting at the postsynaptic site and the termination of its action. The postsynaptic factors involved in the action of 5-HT may be considered at two levels: the postsynaptic receptor site itself and the total postsynaptic response, which depends on the activity of other neurones and may be influenced by other transmitters or drugs affecting them. These mechanisms will now be discussed.

Availability of the 5-HT precursor tryptophan

An important factor controlling how much 5-HT is made in the brain is the availability of its precursor tryptophan, because in physiological conditions tryptophan 5-hydroxylase, the enzyme limiting the rate of 5-HT synthesis, is not saturated with tryptophan (for review see ref. 4).

Administration or deprivation of tryptophan will raise or lower brain 5-HT⁹⁻¹¹ and small changes in plasma tryptophan alter brain 5-HT content¹². After food deprivation^{13,14} or immobilisation¹³ there is an increase in brain tryptophan concentration and increased brain 5-HT turnover. The rise in brain tryptophan is not accompanied by changes in other amino acids¹⁵ and correlates not with total plasma tryptophan¹³ but with the fraction of tryptophan which is not protein bound¹⁶ (free or ultrafilterable¹⁷). Tagliamonte and coworkers have also shown a relationship between free plasma tryptophan and brain tryptophan concentrations^{18,19} and further demonstrated that salicylate, which displaces tryptophan from its plasma albumin binding sites, increases brain tryptophan and 5-HT synthesis²⁰.

The relationship between starvation and brain tryptophan

may be due to the increase in plasma unesterified fatty acids brought about by food deprivation¹⁶. Curzon and colleagues have shown that addition of unesterified fatty acids increases free tryptophan in human plasma *in vitro*. *In vivo*, drugs which alter plasma unesterified fatty acid (UFA) concentrations alter plasma free tryptophan in the same direction²¹. Wurtman's group have found a similar relationship between plasma UFA and free tryptophan²² but dispute the importance of free tryptophan in determining brain tryptophan concentrations and the rate of brain 5-HT synthesis. It is clear, however, that many factors influence the transport of tryptophan into the brain. Insulin release²⁴, ingestion of carbohydrates²⁵, the relative concentration of tryptophan to other plasma neutral amino acids which compete with tryptophan for uptake²⁶, and starvation¹⁵, all affect the amount of tryptophan in the brain. Free tryptophan concentration is clearly therefore only one determinant of brain 5-HT turnover.

Recent work has now shown that hydrocortisone decreases brain tryptophan²⁷ and 5-HT concentrations^{27,28}. This is due to increased activity of the hepatic enzyme tryptophan pyrrolase^{27,28}, which metabolises tryptophan and causes a decrease in the concentrations of free and total plasma tryptophan²⁹ and results in decreased concentrations of tryptophan not only in brain but in liver also²⁹. This indicates another peripheral mechanism altering the availability of tryptophan and therefore brain 5-HT turnover.

Little is known of the processes by which tryptophan passes from plasma into the brain although it has been suggested that these are subject to diurnal rhythms which are accompanied by changes in brain 5-HT metabolism³⁰. Tryptophan transport across synaptosomal membrane has been studied^{31,32} and it has been shown that uptake is stereospecific, temperature dependent and inhibited by metabolic inhibitors, and that there are low and high affinity uptake systems. Several other amino acids compete for transport and exchange diffusion occurs.

Central control of 5-HT synthesis

Glowinski and colleagues reported that 5-HT synthesis was reduced when the intraneuronal concentrations of 5-HT were increased³³⁻³⁵ by monoamine oxidase inhibition. These results and their interpretation are the matter of some dispute^{36,37}.

Chlorimipramine which inhibits the reuptake of 5-HT at the nerve ending^{38,39} decreases the rate of 5-HT synthesis^{40,41}. Since inhibition of reuptake potentiates the effect of 5-HT on 5-HT receptors^{42,43} it may be that increased stimulation of 5-HT receptors causes inhibition of synthesis by a feedback mechanism. Modigh demonstrated that tryptophan hydroxylation was inhibited following chlorimipramine, possibly through an action on nerve impulse flow⁴⁴ in accord with observations that the hydroxylase is influenced by nerve impulse flow^{45,46}. It has been shown that increased receptor stimulation does inhibit impulse flow in central 5-HT neurones⁴⁷. Since imipramine

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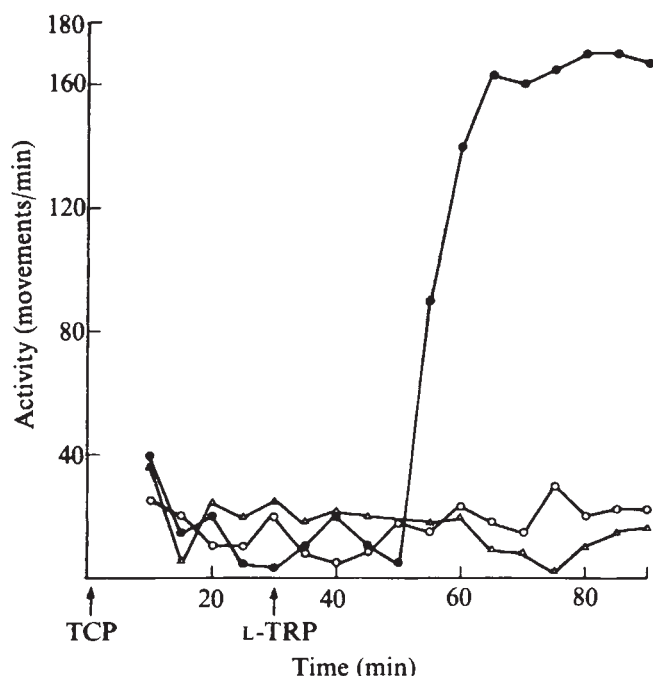


Fig. 1 Typical hyperactivity response in rats after administration of tranylcypromine (20 mg kg^{-1}) followed 30 min later by L-tryptophan (100 mg kg^{-1}) (●). Tranylcypromine administration only (○). Pretreatment with *p*-chlorophenylalanine (300 mg kg^{-1}) on two consecutive days before administration of tranylcypromine and tryptophan (△). Experimental details given in ref. 49.

inhibits tryptophan uptake by synaptosomes⁴⁸, however, some direct effects of chlorimipramine on 5-HT synthesis cannot be ruled out.

Functional activity of brain 5-HT

Our work was aimed at an understanding of how brain tryptophan concentration might control the rate of 5-HT synthesis and its functional activity. When L-tryptophan is administered to a rat there is a small increase in brain 5-HT concentration, but a large increase in the production of the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), indicating an increase in the rate of synthesis of 5-HT, which is then broken down by MAO. No gross behavioural changes occur. Administration of the same dose of tryptophan to rats pretreated with tranylcypromine, a MAO inhibitor, produces a large rise in brain 5-HT. This dependence of the rate of 5-HT synthesis on brain tryptophan concentrations is now well documented.

The increase in 5-HT is associated with characteristic behavioural changes, one of which is hyperactivity which can be measured on activity meters (Fig. 1)⁴⁹. Appearance of the hyperactivity syndrome is dependent on MAO inhibition, tryptophan administration and tryptophan hydroxylase and 5-hydroxytryptophan decarboxylase activity (inhibition of either of these two enzymes with *p*-chlorophenylalanine and 4-bromo-3-hydroxybenzylamine respectively, abolishes the hyperactivity (Fig. 1 and ref. 49)).

There is a positive correlation between the rate of accumulation of brain 5-HT and the rate of development of the hyperactivity, which suggests that the syndrome is produced by 5-HT⁴⁹. Jacobs and colleagues have also demonstrated that the syndrome is the result of 5-HT stimulation^{50,51}. Their studies⁵² have shown that many of the behavioural aspects of the syndrome are present in the mesencephalic rat indicating that these behavioural changes are mediated by the B_1 - B_6 group of neurones, which includes the pontine and medullary raphe nuclei and their synaptic contacts in the lower brainstem and spinal cord.

Our own recent biochemical studies⁵³ have strengthened the

view that it is 5-HT and not a derivative that is responsible for the behavioural change, and we do not believe tryptamine is involved. The hyperactivity following tranylcypromine and tryptamine administration is inhibited by reserpine pretreatment⁵⁴, whereas that produced by tranylcypromine and L-tryptophan, far from being inhibited, has a more rapid onset following pretreatment with either reserpine or tetrabenazine, which block the storage or binding capacity of the synaptic vesicle⁴⁹.

The results of these experiments have been interpreted as follows (see also ref. 55). When tryptophan alone is given, the extra 5-HT synthesised is prevented from spilling over into functional activity by intraneuronal binding and oxidative deamination. When MAO is inhibited the extra 5-HT accumulates at postsynaptic receptors, resulting in the hyperactivity syndrome. When intraneuronal binding is decreased by previous administration of reserpine or tetrabenazine and then MAO inhibited and tryptophan given, hyperactivity is enhanced because the "buffering" effect of vesicular binding against excess 5-HT production has been removed and more 5-HT "spills over" into functional activity.

This interpretation of the data has other implications. First, it suggests that the availability of tryptophan and the rate of 5-HT synthesis may not be very important in the fine regulation of the functional activity of 5-HT. Clearly the availability of tryptophan alters brain 5-HT metabolism but it seems unlikely that it is crucial in controlling the moment to moment functional activity of brain 5-HT. If synthesis were the only factor regulating the functional activity of 5-HT then it would follow that for each molecule of 5-HT released, a molecule would have to be synthesised and synthesis would therefore have to be critically and finely regulated from millisecond to millisecond to control synaptic events.

The second implication is that intraneuronal metabolism and binding of 5-HT are very important in controlling its functional activity. The third implication is that if a proportion of 5-HIAA is being produced intraneuronally from 5-HT which has not been functionally active then the measurement of 5-HIAA in the cerebrospinal fluid is likely to be of limited value as an index of the functional activity of 5-HT (see ref. 56 and later in the discussion of clinical implications).

Because 5-HT is stored in synaptic vesicles and decreasing this storage capacity alters the functional activity of 5-HT, compartmentation of 5-HT at the nerve ending might play an important role in regulating its functional activity and it has been suggested⁴⁹ that there is a prefunctional or reservoir pool, which nevertheless may be in equilibrium with the functional pool.

Unfortunately, at present the mechanisms involved in controlling the release of 5-HT are largely unknown so that fruitful discussion of this aspect is not possible.

To be able to investigate whether the drugs are acting on pre-synaptic mechanisms or postsynaptically the 5-HT agonist, 5-methoxy-*N,N*-dimethyltryptamine (5-MeODMT) has been used. This compound causes a syndrome of hyperactivity qualitatively similar to that seen after tranylcypromine and tryptophan, but with a different time course⁵⁷. It has therefore been used to bypass the effects of drugs on 5-HT synthesis, compartmentation or release and to investigate postsynaptic events.

Effects of lithium, diphenylhydantoin and chlorimipramine

Pretreatment of rats with one injection of either lithium⁵⁸ or diphenylhydantoin⁴¹ causes the hyperactivity following tranylcypromine and L-tryptophan to begin earlier. There was no increase in 5-HT synthesis following one injection of either drug nor any change in response to 5-MeODMT indicating that postsynaptic responses were unaltered. Continued treatment with either of these two drugs caused an increase in 5-HT synthesis rate of 70% (lithium) and 100% (diphenylhydantoin).

It has been proposed that these two drugs may alter the com-

partmentation of 5-HT at nerve ending^{58,59} thereby increasing the amount of 5-HT available for release and functional activity and that then leads to the increased rate of 5-HT synthesis. There is recent indirect evidence that diphenylhydantoin⁶⁰ (see also ref. 61) and lithium^{62,63} increase brain 5-HT turnover in humans.

Inactivation of released monoamine by reuptake into the presynaptic nerve ending is well established^{64,65}. We wished to see whether treatment with a known inhibitor of 5-HT reuptake would alter the hyperactivity syndrome. Pretreatment of rats with chlorimipramine before tranylcypromine and tryptophan caused the hyperactivity to begin earlier⁴¹. This may be because 5-HT cannot be inactivated by reuptake, and the concentration at the postsynaptic receptor site is therefore increased. One injection of chlorimipramine produced changes in the hyperactivity syndrome identical to those seen after one injection of diphenylhydantoin or lithium. But injection of chlorimipramine (25 mg kg⁻¹) twice daily for 2 d, with measurement of 5-HT synthesis on day 3 produced a 60% inhibition of 5-HT synthesis⁴¹ in close agreement with a previous report⁴⁰, in contrast to the increased 5-HT synthesis seen after diphenylhydantoin or lithium. These findings suggest that diphenylhydantoin and lithium are not acting by inhibiting the reuptake of 5-HT.

Postsynaptic events

Finally we come to postsynaptic events as mechanisms for controlling 5-HT functional activity. Increasing the concentration of the inhibitory neurotransmitter γ -aminobutyric acid (GABA) in the brain decreases the hyperactivity resulting from tranylcypromine and either tryptophan or 5-MeODMT administration⁶⁶.

Similarly, depletion of brain dopamine inhibited the hyperactivity from these two treatments⁶⁷. Two interpretations can be made of this. Either a group of dopaminergic neurones lies between the 5-HT neurones responsible for initiating the hyperactivity response and those mechanisms responsible for its behavioural expression⁶⁷ or a group of dopaminergic neurones modulates the through-put from the activation of postsynaptic 5-HT receptors to the brain mechanisms responsible for the hyperactivity. It seems that this dopaminergic system may be involved in regulating the magnitude of the 5-HT behavioural response⁶⁸.

It had previously been shown that chlorpromazine inhibited the 5-HT induced hyperactivity without altering the rate of 5-HT accumulation⁶⁷. It also inhibited the hyperactivity following 5-MeODMT. This suggested that it was acting at a postsynaptic site, probably by blocking 5-HT receptors⁶⁷. Since there is good evidence that chlorpromazine blocks dopamine receptors^{69,70} it now seems reasonable to suggest that it is inhibiting hyperactivity by interfering with the function of the dopaminergic mechanism, discussed above. Furthermore, recent studies (A. R. G. Heal, Boullin and D. G. G.-S., unpublished) have shown that other neuroleptic drugs, haloperidol, spiroperidol and α -flupenthixol (but not the clinically inactive isomer β -flupenthixol) also block 5-HT induced hyperactivity. All these drugs (except β -flupenthixol) also block the locomotor activity produced by a MAO inhibitor and L-dopa, strengthening the view that these drugs inhibit 5-HT responses by their action on dopaminergic neurones, though a direct effect to block 5-HT receptors has not been excluded.

Recently, we have found evidence that the sensitivity of the postsynaptic 5-HT receptor may be altered by electroconvulsive shock⁷¹. While the short term changes of brain 5-HT metabolism seen in rats after a single electroconvulsive shock⁷¹⁻⁷³ cannot explain its therapeutic effect in humans, repeated electroconvulsive shock produces enhanced behavioural responses following 5-HT receptor stimulation. This may be because of a change in the sensitivity of the dopaminergic system involved in the 5-HT hyperactivity syndrome since postsynaptic dopaminergic responses are also enhanced by this treatment^{71,74}.

There is strong evidence that thyrotropin releasing hormone (TRH) over a restricted dose range, causes an enhancement of

the hyperactivity that follows administration of tranylcypromine and L-tryptophan⁷⁵. The effect was seen in hypophysectomised rats, and so could not have involved the pituitary-thyroid axis; nor did TRH alter the concentrations in the brain of 5-HT, dopamine or noradrenaline, or the rate of accumulation of 5-HT after administration of tranylcypromine and tryptophan. On the other hand, it did enhance hyperactivity after 5-MeODMT, which suggests a postsynaptic action on the response to 5-HT.

TRH has been shown not only to modulate several functions of dopaminergic systems⁷⁶⁻⁷⁸ but to alter responses to several centrally acting drugs^{75,79,81} and both in these and in the case of the hyperactivity syndrome, the administration of inhibitors of protein synthesis seems to act in a way that is generally "opposite" to that of TRH^{80,81}. Since the synthesis of 5-HT itself was not inhibited by the drugs, it seems likely that this is another instance in which alterations in dopaminergic transmission influence the functional activity of 5-HT. Although it has yet to be demonstrated that TRH and the protein synthesis inhibitor cycloheximide act at the same site in the brain, both clinical and experimental evidence so far suggest that TRH may enhance⁸¹⁻⁸⁴, whereas cycloheximide inhibits, the release of dopamine in rat brain⁸¹. TRH is widespread in the brain⁸⁵⁻⁸⁶ and unpublished observations (quoted in ref. 87) suggest that it is present in nerve endings along with a specific enzyme for degrading it, all of which is consistent with a modulatory role.

If polypeptides or proteins do mediate or modulate the actions of brain monoamines, then malfunction of such compounds might be confused with a primary abnormality in monoamine function. We found that although treatment with cycloheximide prevented the appearance of the hyperactivity syndrome after the usual doses of 5-MeODMT, increasing the dose of 5-MeODMT overcame this block⁸⁰. It is just possible that in some psychiatric diseases attributed to primary abnormalities in monoaminergic function and which appear to respond to measures increasing or inhibiting monoaminergic activity, the abnormality lies with polypeptide or protein modulators.

In summary it is proposed that the functional activity of brain 5-HT is controlled predominantly by the following factors: (1) The availability of tryptophan to tryptophan 5-hydroxylase; (2) the regulation of the amount of 5-HT available for release by intraneuronal MAO activity and compartmentation such as vesicular storage mechanisms; (3) reuptake of 5-HT from the synaptic cleft; (4) sensitivity of the postsynaptic receptor; (5) regulation of the total postsynaptic neuronal responses through the influence of neuronal systems utilising other neurotransmitters such as dopamine. These processes are outlined in the hypothetical scheme presented in Fig. 2 with indications of the points at which various drugs are thought to act.

Clinical implications

The ethical problems posed by experimental psychopharmacology in humans are daunting. The introduction of really innovative drug therapy in psychiatric disease involves bridging the gap between experimental neuropharmacology and the manipulation of animal behaviour (which is fairly crude) and the manipulation of the neuropharmacology of the human brain to bring about subtle changes in the mind (which is likely to be a much finer process). What conclusions can be drawn from these studies of 5-HT function in rat brain, as mirrored by manipulation of the hyperactivity syndrome, which might help to bridge this gap?

Tryptophan alone produces an increase in the rate of 5-HT synthesis in both the rat brain and in the human brain (if 5-HIAA levels in cerebrospinal fluid can be relied on⁸⁶), but neither hyperactivity in the rat nor, probably, relief of depression in the human results. A simple lack of brain 5-HT is therefore probably not responsible for the depressive state. Yet tryptophan plus a monoamine oxidase inhibitor produces hyperactivity in the rat and is an effective therapy for depres-

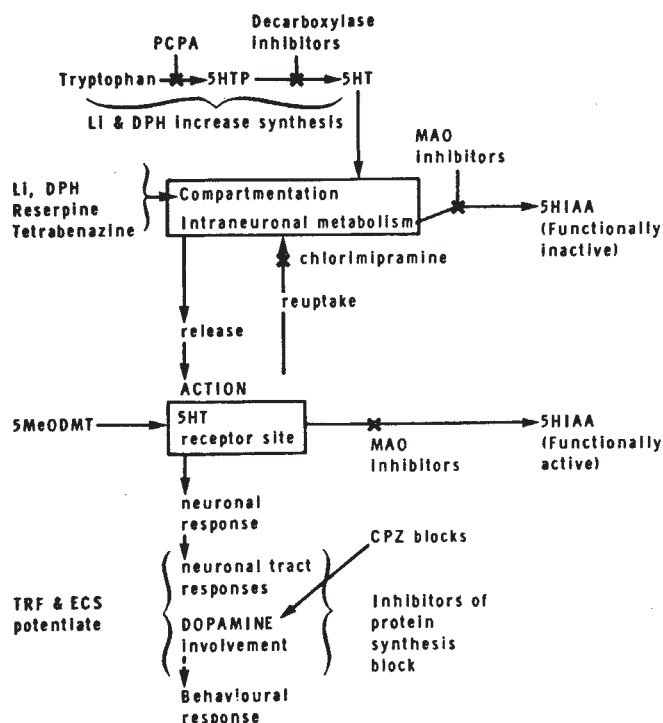


Fig. 2 Some postulated processes regulating 5-HT function and effects of drugs upon them. PCPA, *p*-chlorophenylalanine; 5-HTP, 5-hydroxytryptophan; 5-HT, 5-hydroxytryptamine; MAO, monoamine oxidase; 5-HIAA, 5-hydroxyindoleacetic acid; Li, lithium; DPH, diphenylhydantoin; 5-MeODMT, 5-methoxy-*N,N*-dimethyltryptamine; TRF, thyrotropin releasing factor; ECS, electroconvulsive shock; CPZ, chlorpromazine.

sion⁸⁸. In humans only relatively small doses of tryptophan can be used and MAO cannot be fully inhibited, so the problem is to find other mechanisms for increasing 5-HT function if indeed this is the mechanism by which tryptophan and a MAO inhibitor produce their therapeutic effect. For instance lithium or diphenylhydantoin might be used to render more 5-HT available for functional activity in combination with tryptophan and/or MAO inhibitors. Reserpine or tetrabenazine together with MAO inhibitors and tryptophan if carefully used may prove effective in retarded depression resistant to other forms of therapy⁸⁹. Chlorimipramine, which blocks 5-HT reuptake, may in combination with MAO inhibitors and/or tryptophan also increase 5-HT function fairly selectively and might be used to chart those depressive states amenable to increasing 5-HT function. One might ask, why not just give a 5-HT agonist? 5-MeODMT and *N,N*-dimethyltryptamine while producing the hyperactivity syndrome are potent hallucinogens⁹⁰ but as far as we are aware small sub-hallucinogenic doses have not been tried in depressive states and perhaps the risk is too great. The possibility of more selective 5-HT agonists remains, however.

The similarity between the actions of lithium and diphenylhydantoin on brain 5-HT function prompts us to suggest the trial of diphenylhydantoin in the prevention of manic-depressive diseases. The adverse effects of diphenylhydantoin are well known through its long use in the treatment of epilepsy and they are certainly no worse than those already encountered with lithium. It should also be considered whether when a patient on "prophylactic" lithium for manic-depressive disease goes into a depressive state, MAO inhibitors given in conjunction with lithium might not be the best therapy.

For many years the presence of an endogenous psychotogen in schizophrenia has been sought, sometimes on the assumption that such an endogenous psychotogen could be produced by an abnormal metabolic pathway or by an abnormally functioning

normal metabolic pathway. Because 5-MeODMT, itself an hallucinogen (and which has been invoked as an endogenous psychotogen in schizophrenia^{91,92}), causes the same hyperactivity syndrome as is produced by raised 5-HT levels, we would suggest that abnormal functioning or increased activity of normal transmitters in their normal neuroanatomical pathways could equally be responsible for such changes. The worsening of the schizophrenic state reported after the administration of tryptophan and a MAO inhibitor⁹³ could be interpreted in this light. Since we have seen that postsynaptic mechanisms can change the behavioural response to brain 5-HT, schizophrenia could involve a change in the sensitivity of neuronal responses to "normal" stimuli.

The apparent interplay of dopamine and 5-HT in the production of the hyperactivity syndrome makes one wonder whether in psychiatric states some kind of imbalance occurs between neuronal systems containing 5-HT and dopamine, just as imbalances between cholinergic and dopaminergic systems seem to be involved in Parkinsonism⁹⁴. These speculations are testable clinically using tryptophan, 5-HTP and *L*-dopa, with or without (in the latter two cases) peripheral aromatic amino acid decarboxylase inhibitors.

The finding that chlorpromazine and some other neuroleptics, which it is generally agreed, block dopaminergic receptors in the brain, can probably by this action also block the sequences necessary for the production of the behavioural response to 5-HT, shows that these drugs may not be acting at a primary level, but rather at a secondary level in the therapy of schizophrenic illness. This has already been pointed out⁸. Nevertheless and in the loosest sense, perhaps the phenothiazines are capable of blocking the dopamine gates (and there may be several of them) through which several more primary stimuli may pass. There is no doubt that we need drugs which are not only pharmacologically more specific as agonist-antagonists but also show neuroanatomical specificity of function.

Finally, there is the controversy about the usefulness of 5-HIAA concentrations in the cerebrospinal fluid as an index of the functional activity of 5-HT in humans. This has been dealt with in a comprehensive review⁵⁶. In animal studies 5-HIAA in the cerebrospinal fluid (CSF) does seem to reflect central 5-HT concentrations^{95,96}. Furthermore, drugs affecting 5-HT turnover alter 5-HIAA in the lumbar CSF of human subjects⁹⁷⁻⁹⁹. Against this it has been shown that 5-HIAA injected into the cisterna magna of the cat does not change lumbar CSF 5-HIAA⁹⁹ although this is disputed¹⁰⁰. Furthermore, patients with a spinal block at the thoracic level do not have decreased 5-HIAA^{101,102}. Another study, however, showed substantially decreased CSF 5-HIAA in patients with an incomplete block¹⁰³.

Garelis and coworkers⁵⁶ argue convincingly that CSF 5-HIAA reflects central metabolic changes of 5-HT. If our earlier conclusions regarding the intraneuronal organisation of 5-HT synthesis, compartmentation and metabolism are correct, however, 5-HIAA comes not only from 5-HT that has been functionally active but from 5-HT synthesised in "excess of needs" which has been metabolised by monoamine oxidase intraneuronally. This means that CSF 5-HIAA concentrations include both of these "pools" of 5-HIAA and does not necessarily reflect the metabolism of 5-HT which has been released and has been functionally active.

Until we can measure that proportion of 5-HIAA resulting from the metabolism of functionally active 5-HT (and at present we have no idea under normal situations what this proportion might be) it is premature to conclude that changes in CSF 5-HIAA levels⁵⁶, or rates of rise of 5-HIAA in CSF after probenecid treatment with or without tryptophan loading¹⁰⁴, really indicate the functional activity of 5-HT in the brain. This is not to say that if consistent changes in CSF 5-HIAA are found to occur in certain affective disorders, the knowledge may not be empirically useful; but only to point out the hazards of interpreting the data in terms of the functional activity of 5-HT and its primary aetiological involvement in psychiatric disease.

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The directional dependence of the primary cosmic rays of energies $10^{11} - 10^{12}$ eV

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The apparent sidereal variation in the intensity of muons generated by cosmic rays with energies greater than 10^{11} eV showed a marked change in phase at Northern Hemisphere stations at the time of the recent reversal in sign of the general solar magnetic field. No corresponding change was observed at rather lower energies at Hobart, Tasmania. By adopting a particular model of the large scale interplanetary field, it is possible to account for this behaviour of the sidereal variation in terms of a primary cosmic ray anisotropy which is related to the local direction of the galactic magnetic field.

SYSTEMATIC long term studies of the variations in intensity of muons deep underground have been carried out by a number of investigators to establish the existence of a variation over the sidereal day. The depths at which the observations relevant to this paper have been carried out lie in the range 30-80 m water equivalent (m.w.e.). At a depth of 60 m.w.e. most of the muons derive from primaries with an energy per nucleon of $10^{11}-10^{12}$ eV.

An experimental result that has become rather well established over the years is that in the Southern Hemisphere at Hobart (depth 32 m.w.e.) the sidereal variation has an amplitude of $\sim 0.030\%$ with a time of maximum close to 0600 LST whereas stations in the Northern Hemisphere, Budapest (40

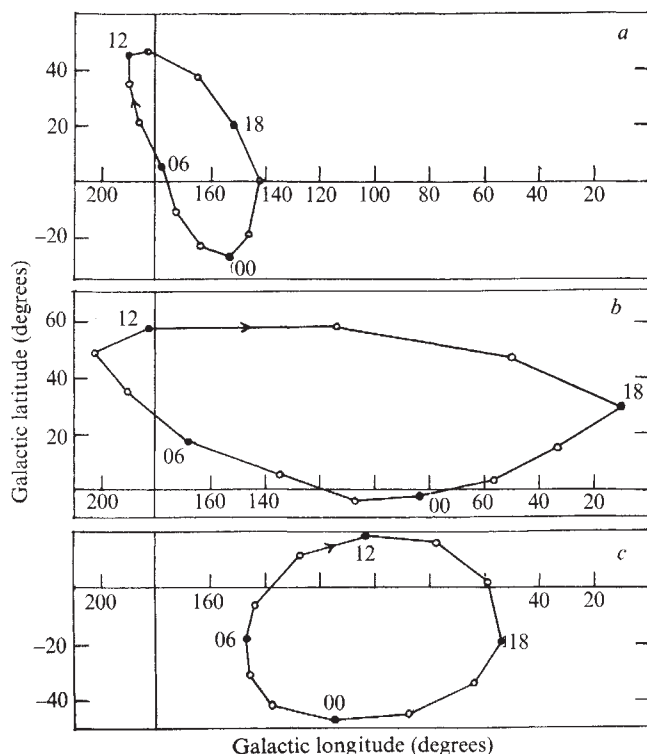


Fig. 1 Galactic scan directions for the vertical direction in London of the epoch 1 interplanetary field configuration. *a*, Average for October–February; *b*, for March–May; *c*, for June–September.

m.w.e.), London (60 m.w.e.), Socorro (80 m.w.e.) and Torino (80 m.w.e.), showed a rather smaller variation with a time of maximum displaced by almost 12 h from that in the Southern Hemisphere. Sekido¹, using a Cerenkov telescope to select high energy muons at ground level which are the progeny of primaries in the same primary energy band as those generating the underground muons, has obtained results which are in substantial agreement with the underground measurements.

Jacklyn² and Sekido¹ have interpreted these experimental results in terms of a 'two-way' anisotropy of the primary cosmic rays in space. That is to say the intensity distribution has two maxima located on the celestial sphere in such a way as to be compatible with the observed times of maximum in the Northern and Southern Hemispheres. This assumes that the directions on the celestial sphere that are scanned by the underground cosmic ray detectors are not significantly affected by the interplanetary magnetic field. In other words the directions in space from which the primary cosmic rays arrive were taken to be those corresponding to the purely geometrical projection of the cone of acceptance of the telescopes on to the celestial sphere after allowing for the (small) deflection in the geomagnetic field. In fact we shall show that the presence of the interplanetary field changes very substantially the scanning directions of underground telescopes at the depths we are considering.

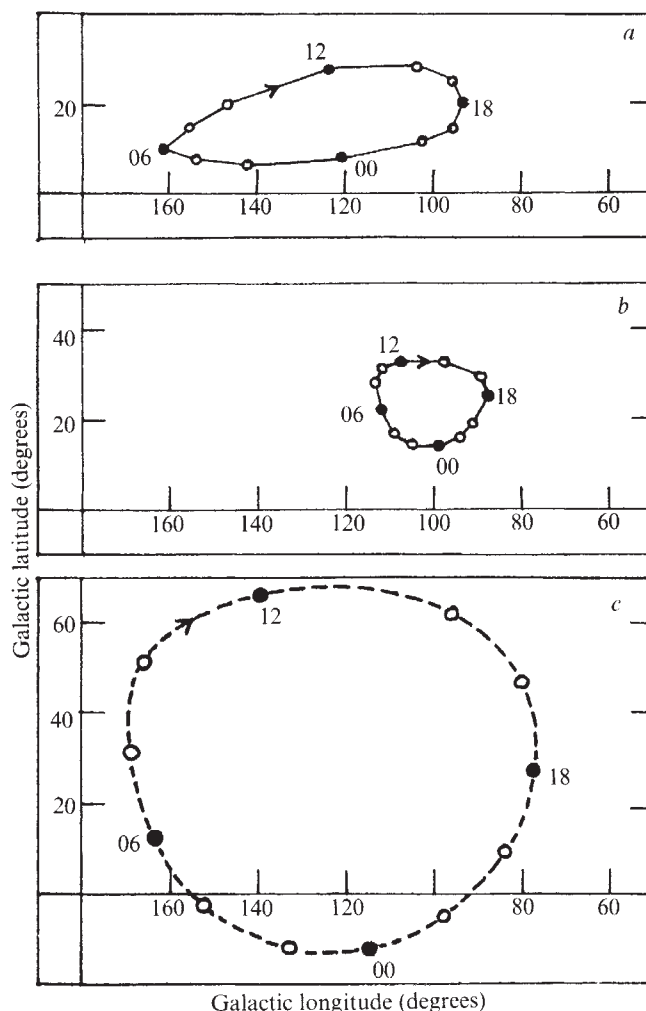
Speller *et al.*³ and Barnden⁴ have examined the effects of an interplanetary magnetic field model consisting of sectors in the meridian plane of alternate polarity on the trajectories of cosmic rays in the range of magnetic rigidities appropriate to muon detectors at 60 m.w.e. In particular Speller *et al.*³ showed that the intensity variation over the sidereal day observed in the Northern Hemisphere could be accounted for by the motion of the solar system relative to the average rotation frame of the Galaxy in our neighbourhood. The cosmic rays are assumed to rotate with the Galaxy and the relative motion of an observer in the solar system then leads to a sidereal variation in close agreement with the observed amplitude and phase.

The variation observed at Hobart could not, of course, be explained in this way since the time of maximum differs by 12 h from that expected for the solar motion effect, and in addition the amplitude is too large.

During the period after 1970 the sidereal variation observed at stations in the Northern Hemisphere changed markedly from that found in the earlier years. Data from the vertical recorders at the Holborn laboratory in London show that the sidereal wave has shifted in phase by $\sim 180^\circ$ so that the time of maximum is now ~ 0600 and has been for the past few years. Similar changes in phase have been observed at Torino by Cini-Castagnoli (personal communication) and by Swinson⁵ for his Socorro station. This change in phase⁶ seems to have taken place at the same time as the reversal of the large scale solar photospheric fields⁷. Neither the solar field nor the sidereal variation itself changed abruptly and, as far as can be seen, the change was spread over several years. For convenience, however, we assume here that the change took place at the end of 1969 and we shall refer to the period before the changeover as epoch 1 and the period from 1970 on, following the changeover, as epoch 2.

The change in phase of the sidereal wave is apparently confined to the Northern Hemisphere stations and has not occurred at Hobart⁸. As concluded by Cini-Castagnoli *et al.*⁶ it is very likely connected with a change in the interplanetary field which is in turn related to the magnitude and sign of the photospheric fields. We have therefore been led to investigate

Fig. 2 Galactic scan directions for the vertical direction in London of the epoch 2 interplanetary field configuration. *a*, Average for April–July; *b*, for remaining months; *c*, scan for infinite energy particles.



the effects on cosmic ray trajectories of a rather different interplanetary field model from that used by Speller *et al.*³ and by Barnden⁴.

The interplanetary field model

It is clear that the changes in the large scale photospheric fields observed by Howard⁷ must produce corresponding changes in the interplanetary magnetic field and evidence for such changes based on *in situ* spacecraft magnetometer measurements has been given by Rosenberg⁸ and by Hedgecock¹⁰. There is no doubt that the presence of the interplanetary magnetic field affects the trajectories of primary cosmic rays of magnetic rigidity $\leq 10^{12}$ V which generate the bulk of the muons monitored at depths < 80 m.w.e. and it is to be expected, therefore, that a substantial change in the field will in some measure influence the character of the sidereal variation.

As stated above the effect on such primaries of an interplanetary field with a 2- or 4-sector meridional structure has been investigated by Speller *et al.*³ and by Barnden⁴ and these authors have shown that this type of field does not scatter the relevant cosmic rays sufficiently to prevent the observation of a galactic anisotropy at the Earth although it does reduce the measured amplitude. It now seems possible, however, that the interplanetary magnetic field is not of this sectorized form, but, instead, derives from a large scale solar dipole in which the field north of the solar equatorial field is predominantly of one sign while that south of the equatorial plane has the opposite sign^{11,12}. The two-sector structure of the interplanetary field, which is the most frequently observed configuration, is then attributable to the tilt angle between the axis of the solar dipole and the Sun's rotational axis.

A field of this configuration has a much more serious perturbing effect on cosmic ray trajectories than one which is meridionally sectorized, where the alternating sign of the field is to some extent self-cancelling so far as the particle deflections are concerned. Consequently there is good reason to suppose that the reversal of such a stretched dipole field would produce a significant change in the form of the cosmic ray anisotropy observed at the Earth.

In determining the directions on the celestial sphere scanned by an underground monitor in the course of the day it is necessary first to compute the trajectories of representative cosmic ray primaries which reach the Earth at the appropriate latitude and longitude for a variety of times of day throughout the year and then combine these with the response functions of the detector^{13,14}. The interplanetary field model we have used is a simple idealised one of the form

$$B_r = B_0 \left(\frac{a}{r} \right)^2$$

$$B_\theta = 0$$

$$B_\phi = B_0 \left(\frac{a}{r} \right) \sin \theta$$

where θ is the heliographic colatitude and B_0 is the radial component of the field at the Earth's orbit ($r = a$). Although, as stated above, the dipole axis must be inclined to the Sun's axis of rotation we do not know what this angle is, and for the purpose of the present calculation it has been taken to be zero. We have also assumed that to a sufficiently good approximation the radial and tangential components of the field at 1 AU can be taken as equal and written as $B_0 = 2.5$ nT. B_0 is positive in sign (the field is directed away from the Sun) everywhere north of the solar equatorial plane and negative everywhere to the south of it for epoch 2. The field in epoch 1 has been taken to be the exact reverse. These field directions correspond in sign to those measured in the photosphere. The trajectory integrations

were terminated at a heliocentric distance of 30 AU, under the assumption that the field is ineffective at greater distances but extending the field model and the integration process to 100 AU does not significantly change the asymptotic directions of the trajectories.

Using these two field models, average viewing directions weighted according to the response function for a depth of 60 m.w.e. at London have been computed at 2-h intervals throughout the sidereal day for each month of the year for the two epochs. Certain months have very similar directional scans and to reduce the number of diagrams these have been combined together. Figures 1 and 2 show, in galactic coordinates, the effective 'direction of look' of the vertical underground telescopes as a function of sidereal time.

As Figs 1 and 2 show, reversal of a stretched dipolar field of the kind used in the trajectory computation has a significant effect on the directions scanned by the Holborn muon monitor in the course of the sidereal day. The corresponding purely geometric scan for infinite energy particles or zero interplanetary magnetic field is shown in Fig. 2 for comparison and it will be clearly evident that if the interplanetary field is of the form assumed it must be taken into account in interpreting the sidereal variation in terms of an anisotropic distribution of cosmic rays in space.

Cosmic ray intensity contours

The radius of curvature of the trajectory of a cosmic ray with magnetic rigidity 3×10^{11} V in the galactic magnetic field of ~ 0.3 nT is $\sim 3 \times 10^{14}$ cm which is very small compared with the dimensions of a spiral arm. It seems highly improbable that the transport mean free path in interstellar space can be much less than 1 pc, and consequently the motion of the primary cosmic rays we are concerned with can be considered to be nearly adiabatic in the galactic field. In these circumstances it is plausible to regard the cosmic ray directional distribution as essentially a pitch angle distribution having symmetry about the local direction of the magnetic field whatever that may be. If this picture is valid we would then expect the angular distribution of the cosmic rays to be symmetrical about the two points on the celestial sphere where it is intersected by the local field vector.

With this assumption of symmetry and the directional scans of Figs 1 and 2, the measured intensity variation over the sidereal day can be used to construct cosmic ray intensity contours for galactic longitudes in the range $0-200^\circ$. In doing so, it is necessary to correct the measured sidereal variation for two separate solar effects.

The first of these is the solar motion effect referred to above which can be calculated from the known solar velocity, the exponent of the cosmic ray spectrum in the relevant energy region and the directional scan diagrams. The second correction arises from the solar diurnal temperature wave in the terrestrial atmosphere which generates a spurious sidereal time variation of the muon intensity because of its seasonal dependence. The variation in the muon intensity underground which is generated in this way is a combination of pion and muon decay effects. The former leads to a positive temperature coefficient whereas the latter has the opposite sign. We have not attempted to calculate this correction from the (uncertain) atmospheric temperature variation but have instead made use of the spurious sidereal variation measured by inclined telescopes situated at the same depth in Holborn with their directions of maximum sensitivity directed along the Earth's axis of rotation. The 'sidereal variation' observed in this way arises entirely from atmospheric temperature variation, since such telescopes do not see any genuine variation from a primary cosmic ray anisotropy.

The intensity contours derived in this way are shown in Fig. 3, and Fig. 4 illustrates the degree of fit attained between the experimental data and these contours. In the latter diagram 2-h departures from the mean intensity over the sidereal day at Holborn averaged for the two epochs and corrected for the

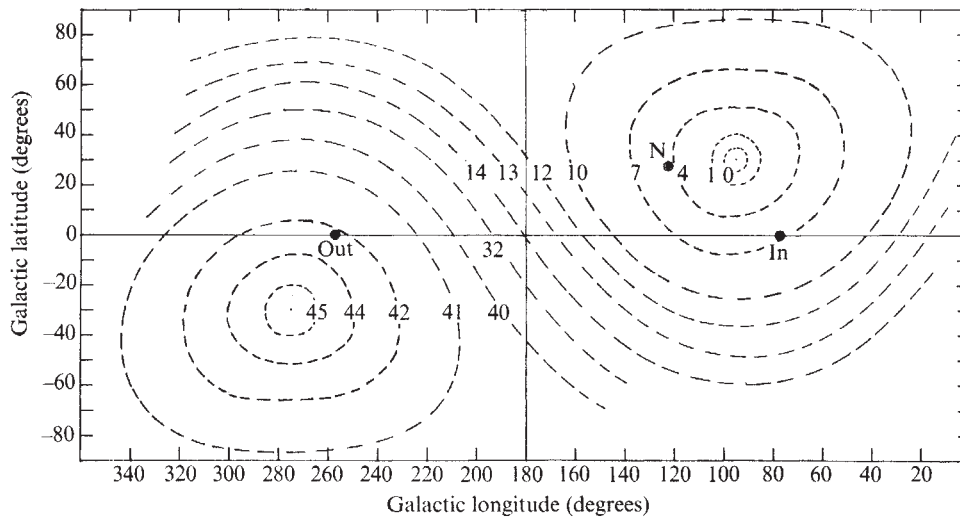


Fig. 3 Intensity contours in galactic coordinates inferred from the sidereal variation observed in London and Hobart. They are in units of 7.5×10^{-5} . 'In' and 'Out' correspond to the local spiral arm in and out directions. N is the direction corresponding to the Earth's North Pole.

two solar effects described above have been plotted together with the variation to be expected on the basis of Fig. 3.

To extend the contours derived from the London data to cover the complete range of galactic longitudes we have made use of observational data from Hobart together with galactic scan diagrams for that station, similar to those in Figs 1 and 2. It turns out that while the Hobart scans extend the range of longitudes covered beyond 200° , they also show considerable overlap with those for London, which permits a satisfactory patching together of the data. Figure 5 illustrates the degree of fit between the Hobart measured first harmonics and those deduced from the intensity contours of Fig. 3 for the two epochs. It is of particular interest to note that reversal of the interplanetary magnetic field does not reverse the phase of the sidereal variation in Hobart as it does in London.

Conclusions

In summary we believe that the observational data on the sidereal variation of cosmic rays in the energy range 10^{11} – 10^{12}

eV in both the Northern and Southern Hemispheres, including the phase reversal in the former, are all compatible with the intensity distribution shown in Fig. 3, provided that the field configuration in the interplanetary field model correctly describes the magnetic conditions in interplanetary space. The axis of symmetry of the cosmic ray distribution, which on this model should correspond to the local direction of the interstellar magnetic field, lies at $\sim 25^\circ$ to the Earth's axis of rotation. It is fortunate that the angle is not smaller, since the possibility of observing an anisotropy of the kind discussed here diminishes rapidly as this angle tends to zero, even for high energy particles where the effect of the interplanetary field is insignificant.

It has often been argued that the presence of the interplanetary magnetic field makes it impossible to observe any anisotropy for primary cosmic rays in this range, and that because of this, it is necessary to make such measurements at much higher energies, where the effect of the field on the particle trajectories is negligible. Our result is somewhat ironic in that the presence of the interplanetary magnetic field apparently results in a more favourable directional scan for the lower energy particles than for high energies because of the small angle between the Earth's axis and the axis of symmetry of the cosmic ray distribution.

Fig. 4 Corrected average 2-h departures of the cosmic ray intensity from the mean in London for epochs 1 (a) and 2 (b) together with the variation (dotted curves) expected from Fig. 3.

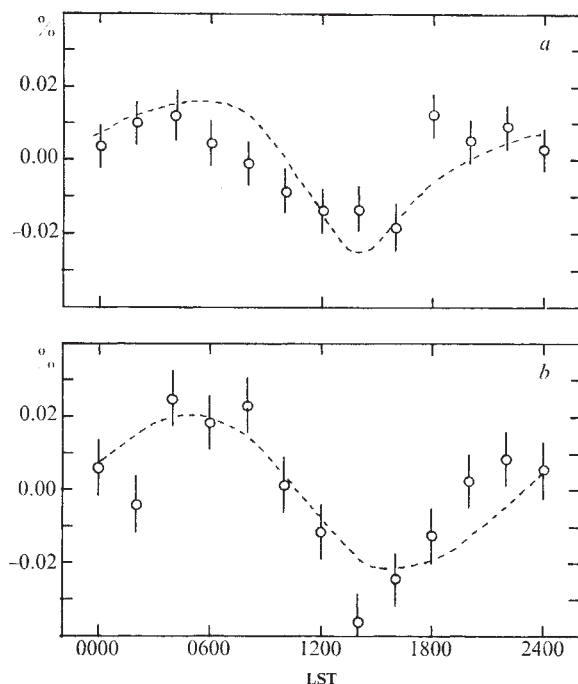
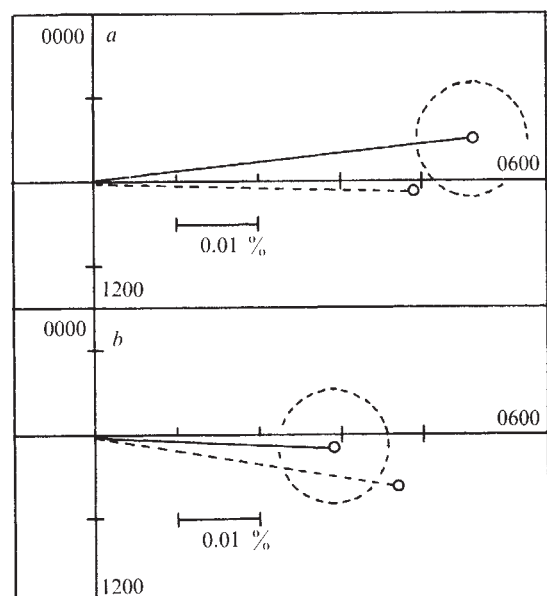


Fig. 5 Harmonic dials showing the 24-h sidereal waves to be expected from Fig. 3 (---) together with those measured in Hobart (—) for epochs 1 (a) and 2 (b).



The question as to whether an apparent sidereal anisotropy can be simulated by purely local solar effects (see for example Swinson¹⁶) has often been discussed in the past and in this connection it should be borne in mind that the axis of symmetry of the distribution in Fig. 3 lies along the normal to the ecliptic, so that any preferential solar induced streaming of the cosmic rays perpendicular to the ecliptic plane could in principle produce an angular distribution similar to that described here. It does not, however, seem possible to reconcile other established characteristics of the sidereal variation with such a solar effect. In particular we are not aware of any solar process which is capable of explaining the change in phase of the sidereal variation observed at Northern Hemisphere stations in 1969–70 together with the absence of any corresponding change at Hobart. In the absence of any satisfactory explanation based on solar processes alone it is our opinion that the angular distribution shown in Fig. 3 is most readily interpreted in terms of a galactic magnetic field pitch angle distribution.

Because of the low statistical accuracy of the measured sidereal variation, the uncertainty of the corrections applied, the relative insensitivity of the observational method which is a consequence of the small angle between the axis of symmetry of the intensity distribution and the Earth's rotational axis and, above all, the uncertainty in the interplanetary field model, the contours of Fig. 3 cannot be more than a very rough approximation to the truth.

Accepting, however, that within rather wide limits of uncertainty the intensity distribution is valid, a plausible explanation for such a distribution can be given. The intensity minimum of Fig. 3 is taken to correspond to the galactic magnetic

field direction leading inwards towards the centre of the Galaxy, a not unreasonable assumption since it lies closest to the 'spiral in' direction of the local arm. If, then, cosmic rays of energy $> 3 \times 10^{11}$ eV are predominantly of extragalactic origin, this pitch angle distribution could be the result of the extra amount of galactic material traversed by particles whose trajectories are such that they arrive at the Earth after reflection at some point in the spiral arm closer to the galactic centre than the Solar System and where the magnetic field is stronger than it is in the neighbourhood of the Sun. An additional path length corresponding to the traversal of ~ 0.3 g cm^{-2} of interstellar matter would suffice to explain the observed departure from isotropy.

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Genomic transcriptional activity and the structure of chromatin

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Nucleic acid hybridisation has shown that micrococcal nuclease-derived chromatin subunits from the cells of Xenopus laevis contain fragments of ribosomal 28S, 18S and 5S RNAs within the population of 200 base-pair pieces of DNA. Subunits from cultured embryonic cells actively transcribing ribosomal RNA contain only 70–74% of the cistrons present in undigested wild-type DNA, while subunits from adult erythrocytes not active in RNA transcription contain close to 90% of the ribosomal cistrons in native chromatin.

CHROMATIN can be cleaved, either by endogenous nucleases¹ or by exogenously supplied micrococcal nuclease^{2,3,7} into fragments containing discrete size classes of protected double-stranded DNA. Noll² has reported that about 85% of rat liver chromatin is susceptible to such digestion giving rise to a basic "chromatin subunit" with a sedimentation value of 11.2S and containing about 205 base pairs of DNA associated with nucleoproteins. Kornberg⁴ has proposed a model for chromatin structure based on such DNA subunits being associated on the outside of an octamer of histones^{8,9} with the composition f2a₁₂ f3₂ f2a₂ f2b₂. Van Holde¹⁰ has proposed a similar chromatin model. Histone f1 is not present in these subunits³⁻⁷. Electron

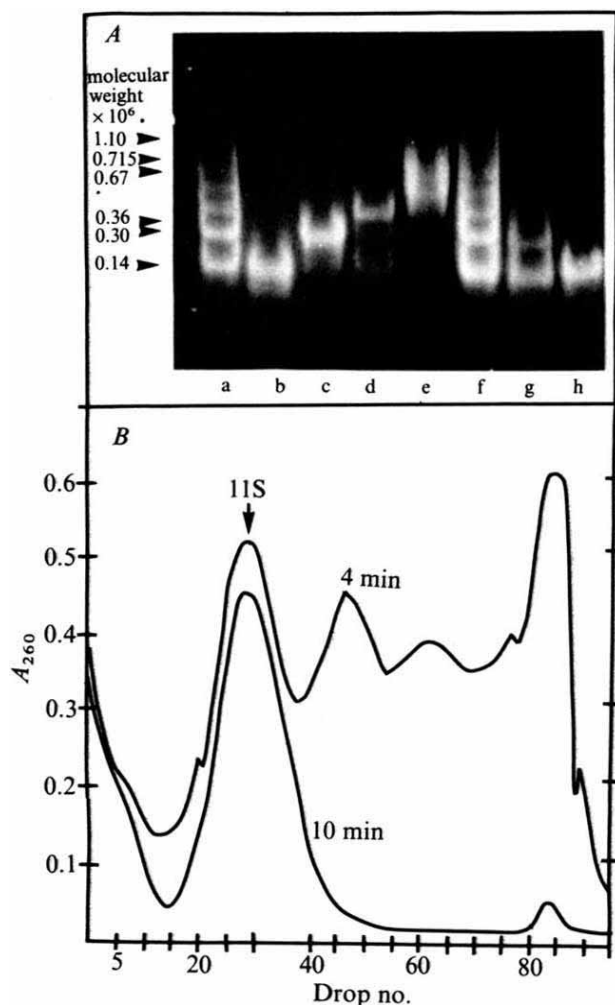
microscopic¹¹⁻¹⁴ and neutron diffraction studies¹⁵ have strongly supported such a beads-on-a-string model for chromatin. Little is known, however, about the functional significance of such a chromatin substructure. It is thus interesting to ask whether the areas of chromatin that are more accessible to various chemical probes, such as nucleases¹⁶⁻²¹, are also the areas more active in genetic transcription. And, conversely, to query whether those areas of chromatin protected from ready nuclease digestion represent less active, or perhaps, repressed parts of the genome²². We present here results of nucleic acid hybridisation experiments in which purified ribosomal RNAs (rRNAs) have been annealed to the DNA from chromatin subunits prepared by micrococcal nuclease digestion of isolated nuclei. Nuclei were obtained from tissue culture cells actively transcribing the ribosomal genes and from mature erythrocytes, which are not active in transcription. By comparing the susceptibility to nuclease digestion of DNA in both transcriptionally active and inactive states we hoped to obtain information concerning the structure and function of native chromatin.

Isolation of chromatin DNA fragments

Nuclei isolated from embryonic *Xenopus laevis* tissue culture cells and treated with micrococcal nuclease (EC 3.1.4.7) for 4 min at 37 °C release, when lysed, pieces of chromatin that are integral multiples of a discrete particle size, the monomer

Fig. 1 A. Patterns obtained when DNA fragments were separated by electrophoresis on a 1.4% Agarose slab gel, stained with ethidium bromide and photographed under ultraviolet light using the methods of Sharp *et al.*⁴¹. The electrophoresis buffer contained 40 mM Tris base (pH 7.8), 5 mM sodium acetate and 1 mM EDTA. Electrophoresis towards the anode was for 2 h at room temperature on a Raven slab gel apparatus run at a constant 35 mA. Slot a: 15 μ g of DNA fragments isolated from tissue culture cell nuclei that had been incubated with micrococcal nuclease (500 U ml⁻¹) for 4 min

at 37 °C; Slots b-e: DNA fragments isolated from tissue culture cell monomer, dimer, trimer and tetramer (and larger) chromatin subunits separated by centrifugation on a 10–30% sucrose gradient run for 20 h at 30,000 r.p.m. in a Beckman ultracentrifuge using a SW 50.1 head; slots b-e contained, respectively, 10 μ g, 10 μ g, 5 μ g, and 10 μ g of DNA each. Slots f-h: sequence showing DNA fragments from timed nuclease digestions of tissue culture cell chromatin; slot f: 4-min digest of nuclei (30 μ g DNA); slot g: 8-min digest (10 μ g of DNA); slot h: 10-min digest (10 μ g of DNA).



subunit. The chromatin subunits have a sedimentation value of about 11S when run on a sucrose gradient (Fig. 1). The double-stranded DNA isolated from these monomers has a molecular weight of about 1.4×10^5 as judged by its comigration during electrophoresis with marker viral SV40 DNA endonuclease restriction pieces of known molecular weight^{23,24}. In Fig. 1A, columns a and f show the Agarose gel electrophoretic pattern of DNA fragments isolated from nuclei digested with nuclease for 4 min at 37 °C. On the left of the figure are the molecular weights of marker SV40 DNA pieces electrophoresed on the same slab gel. In the 4-min digest there are at least six DNA bands visible under ultraviolet light in this slab gel stained with ethidium bromide. These bands are, as shown by the molecular weight markers, integral multiples of the approximately 200 base pairs present in the monomer DNA. As columns g and h show, with increasing times of nuclease digestion, the larger multimeric DNA bands disappear from the profile so that by 10 min of incubation (column h) 70–75% of the bulk DNA in these tissue culture cells has been converted to the 140,000-dalton monomer DNA size. Such 10-min digests of culture cell chromatin were thus routinely used as a source

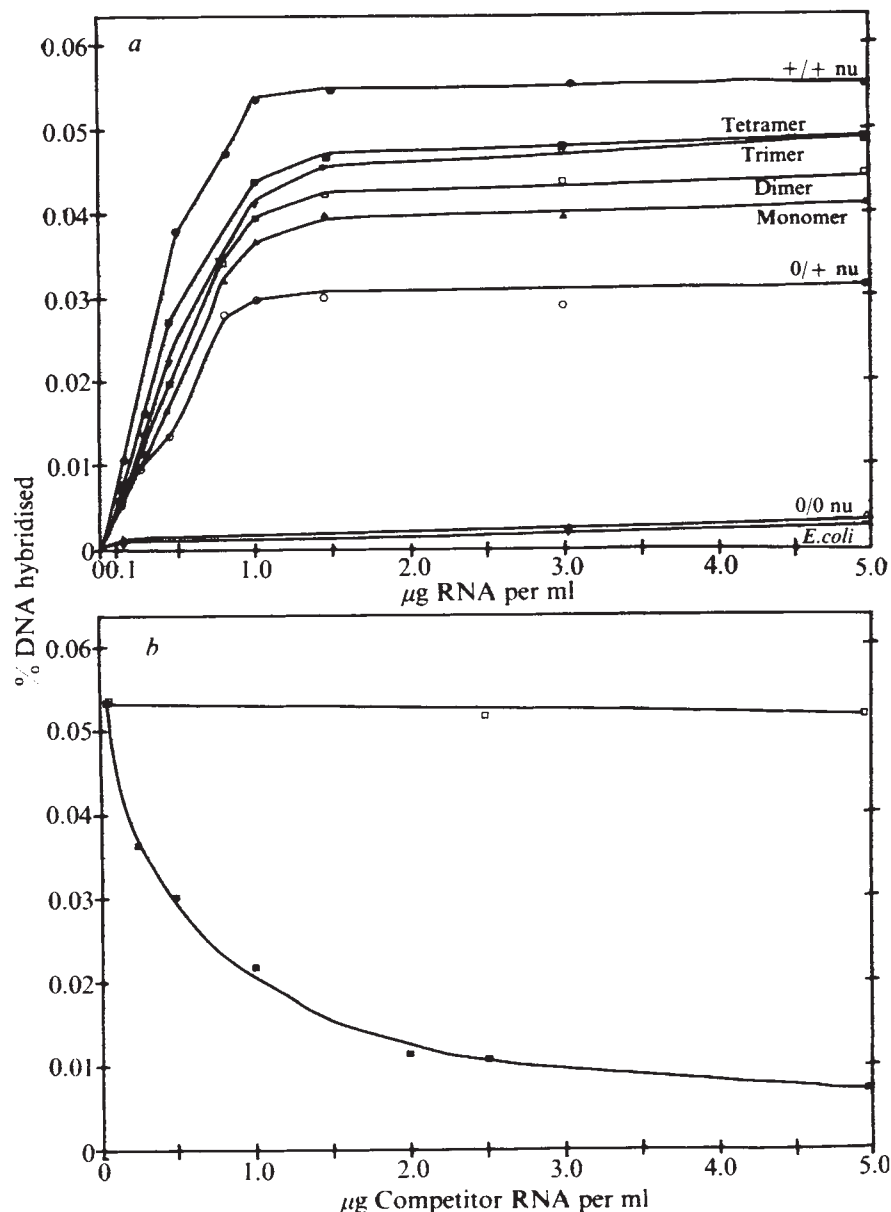
B, Absorbance profile (at 260 nm) of chromatin subunits from actively growing tissue culture cells separated on a 10–30% sucrose gradient as described in (A). The sucrose solutions contained 0.2 mM EDTA and 2.85 mM phenylmethylsulphonylfluoride (PMSF). After centrifugation the gradients were pumped through a Uvicord II recording UV analyser and separated into fractions by drops. The upper curve gives the profile from a 4-min nuclease digest (at 37 °C) and the bottom curve the profile of a 10-min digest of tissue culture cell chromatin. Catalase, as a convenient marker for the 11S region, was run on a separate gradient. For preparation and micrococcal nuclease digestion of nuclei, exponentially growing tissue culture cells were collected by standard procedures⁴² and washed in homogenisation solution (10 mM Tris-HCl, pH 7.8, 1 mM MgCl₂, 0.05 M NaHSO₃, 0.15 M sucrose) at 4 °C. The cells were then centrifuged at 250g for 10 min and the pellet was resuspended in hypotonic homogenisation solution (above buffer without sucrose) and swollen for 10 min at 4 °C. After further pelleting the swollen cells were resuspended in complete homogenisation solution containing 0.5% Triton X-100 and pipetted vigorously. After washing and pelleting the isolated nuclei were resuspended in homogenisation medium (without Triton X-100) containing 1 mM CaCl₂. Micrococcal nuclease was added to the solution (with concentrations of nuclei of up to 5×10^8 per ml) to give a final nuclease concentration of 300–500 U ml⁻¹ depending on the experiment. The nuclei were nuclease digested at 37 °C for the times indicated and then pelleted at 6,000g for 10 min. Erythrocyte nuclei were isolated by the procedures of Destree⁴³. Chromatin particles were freed from the pelleted nuclei by homogenisation in the complete homogenisation solution containing 0.2 mM EDTA and 2.85 mM PMSF at pH 8.0 and 4 °C. Nuclear membranes and insoluble materials were removed by centrifugation. The chromatin-containing supernatant was then concentrated and dialysed against the appropriate buffer solutions for a particular experiment. Protein determinations were either by the method of Lowry⁴⁴ or by the use of the E_{280}/E_{260} ratio using appropriate standards. To isolate DNA, whole chromatin or chromatin subunits derived by nuclease digestion were dialysed against SSC (0.15 M NaCl, 0.015 M trisodium citrate, pH 7.8) before extraction of DNA by the technique of Brown and Weber^{27,28}.

of monomer DNA for most of the hybridisation studies reported here.

In all of these DNA preparations from nuclease-digested chromatin a slight smear of DNA fragments, with a few weak but distinct minor bands, electrophoresed faster than the major monomer DNA band. These bands do not show up very well on this photograph but were always a very minor fraction of the total DNA present in any preparation.

When the chromatin fragments from a 4-min digest of culture cell nuclei were centrifuged on a 10–30% sucrose gradient the absorbance profile of the gradient shown in Fig. 1B was obtained. As is evident, it is possible to separate the oligomeric chromatin fragments of the digest according to increasing size and weight on such gradients. The monomeric subunit migrates with a sedimentation velocity of about 11S as determined by using a catalase marker. Figure 1B also shows the absorbance profile of chromatin from a 10-min digest confirming that by this length of nuclease treatment virtually all the remaining DNA of the culture cells is of the monomer DNA size class. When the DNA from each of the gradient peaks from a 4-min chromatin digest are extracted and electrophoresed on an

Fig. 2 a, Saturation profiles for hybridisations obtained from one experiment in which all DNA-containing filters were annealed in identical conditions with increasing concentrations of the same batch of purified ^{32}P -18S + 28S rRNA. The specific activity of the RNA was 728,000 c.p.m. per μg RNA. Monomer, dimer, trimer and tetramer (and larger) DNAs were isolated from logarithmically growing tissue culture cells as described in the legend to Fig. 1. Embryos of +/+ nu, 0/+ nu and 0/0 nu genotypes were obtained by mating two heterozygous anucleolate mutant frogs by standard techniques⁴⁵, their DNAs were isolated as described. *E. coli* DNA was from Sigma. Curve codes as labelled. Each point is the average of the counts on two separate filters. Counts on filters were corrected for non-specific binding to blank filters (about 30 c.p.m.) and for loss of DNA from filters during the hybridisation. Each 6-mm filter initially contained 5–10 μg of immobilised DNA and during the annealing reaction no more than 10% of the DNA was lost from the filters. Loss was monitored by counting the radioactivity left after incubation on test filters to which high specific activity ^{14}C -DNA from *Xenopus* culture cells had been added. Hybridisation conditions were $2\times\text{SSC}$, 50% formamide, 45 °C, 16 h. **b**, Competition hybridisation curves obtained when increasing concentration of 'cold competitor' rRNA was added to an annealing mixture containing a constant amount (1.5 μg) of ^{32}P -18S + 28S ribosomal RNA from *Xenopus* tissue culture cells. Conditions were as in (a) except that the specific activity of the ^{32}P -rRNA was 3.4×10^5 c.p.m. per μg RNA. ■, Unlabelled competitor *Xenopus* 18S + 28S RNA; □, ^{32}P -18S + 28S RNA. For hybridisation, whole or monomer DNA was prepared as described in the legend to Fig. 1. DNA determinations were by the method of Burton⁴⁶. Whole, undigested DNA was sheared by either repeated passages through a 26 gauge needle or by homogenisation in a Virtis S23 homogeniser at full power for 1 min. Nuclease-derived DNA fragments were not sheared. For hybridisation experiments the membrane filter technique described by Gillespie³⁴ was used. The purified DNA dissolved in $1/10\times\text{SSC}$ at 100 μg per ml was heat denatured at 95 °C for 15 min. The denatured solution was cooked quickly in a dry-ice acetone bath and diluted with cold $6\times\text{SSC}$ to a concentration of less than 10 μg per ml. The $6\times\text{SSC}$ -DNA solution was made 0.05 M in KOH and 1 mM in MgCl_2 to increase the retention of small DNA fragments to nitrocellulose filters. Millipore HA filters (45 mm) were washed with hot (90 °C) $6\times\text{SSC}$, cooled and then loaded with the ice-cold single-stranded DNA solution by slow gravity filtration at 4 °C. Gravity filtration was repeated twice. The loaded filters were then treated for 1 h at room temperature with Denhardt's⁴⁷ preincubation mixture, dried and baked overnight at 60 °C. From each 45-mm HA filter, small 6-mm mini-filters (each containing 5–10 μg of DNA) were cut. After annealing of DNA filters with radioactive RNA as described, the RNA-DNA hybrid-containing filters were washed extensively with $2\times\text{SSC}$ followed by incubation for 15 min at 37 °C in $2\times\text{SSC}$ containing boiled RNase (50 μg ml⁻¹). The filters were then dried and counted in an Isocap scintillation counter. All counts on filters were corrected for background and for channel spill over where two isotopes were used. To purify isotopically labelled RNAs, exponentially growing tissue culture



cells in low phosphate medium were labelled for 3 d with ^{32}P as orthophosphate (New England Nuclear) and then chased for 48 h. Alternatively, cells in normal medium were labelled similarly with ^3H -uridine (New England Nuclear). Whole cell RNA was extracted and separated into fractions by polyacrylamide gel electrophoresis as described by Miller and Knowland³³. Segments of the gels corresponding to either the 28S ribosomal RNA alone or to the 18S + 28S rRNAs together were cut from the gels. The RNA was recovered from these gel fragments by electrophoresis against a dialysis membrane followed by ethanol precipitation. Radioactive 5S RNA, on the other hand, was recovered from the polysome fraction of ^{32}P -labelled tissue culture cells. RNA was extracted from polysomes and separated by gel electrophoresis as described above. The 5S RNA was further purified by chromatography on a Pasteur pipette column containing methylated albumin kieselguhr using a 0.2–1.5 M NaCl gradient for development⁴⁸.

Agarose slab gel the results shown in columns b–e of Fig. 1 are obtained. As expected, the peaks on the gradient correspond primarily to DNA fragments of increasing molecular weight. There is, however, always some minor cross contamination of all of the gradient-separated DNA bands. Nevertheless, by sucrose gradient separation it is possible to obtain greatly enriched populations of chromatin particles of a particular size class for extraction of DNA fragments for hybridisation experiments. The same experiments have also been done with DNA fragments of various sizes isolated by elution from

Agarose gels after electrophoretic separation. The annealing results are similar in both cases.

Hybridisation with DNA from transcriptionally active cells

The reiterated ribosomal cistrons of *X. laevis* comprise about 0.2% of the nuclear DNA of diploid somatic cells^{25,26}. This corresponds to 450–500 adjacent sets of ribosomal genes per haploid genome^{25–27}. Each of these tandemly repeated gene sets, in turn, includes DNA which codes for a precursor to

18S and 28S ribosomal RNA as well as DNA for the untranscribed 'spacer' regions of high deoxyguanylic and deoxycytidylic acid (G+C) content^{28,29}. Thus the DNA from wild-type animals contains about 1,000 sets of these ribosomal cistrons per diploid genome. Within the cell nucleus of such wild-type animals there are usually two nucleoli (hence they are +/+ nu) with each nucleolus containing about half of the total ribosomal cistron sets localised to a nucleolar organiser region of one of the chromosomes³⁰. There is also a stable mutation, the anucleolate mutation³¹ known in certain strains of these animals which involves the total deletion of the

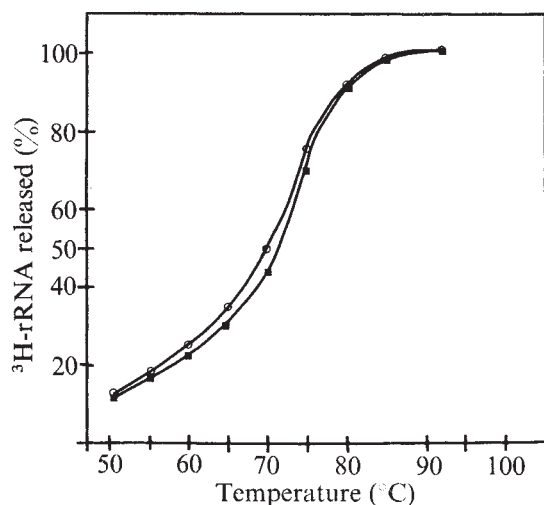


Fig. 3 Thermal dissociation profiles of rRNA-DNA hybrids of sheared whole DNA (○) and nuclease-derived monomer DNA (■) from actively growing tissue culture cells. Filters containing (28S+18S)RNA-DNA hybrids were heated in 1 ml of 0.1×SSC for 5 min at each temperature, and the radioactivity released was measured after precipitation with cold trichloroacetic acid. At no temperature were the ³H counts released from the filters able to rebind to other membrane filters, showing that intact hybrids were not released³⁴. Line codes as labelled.

reiterated cistrons from the normal nucleolar organiser region of the chromosome³⁰. Animals heterozygous for this mutation (+/0 nu) contain only half the normal complement of ribosomal cistron sets and have only one nucleolus per diploid somatic cell. Individuals homozygous for the anucleolate mutation (0/0 nu) have no detectable ribosomal 18S and 28S cistron sets³⁰ and die at an early larval stage. DNA isolated from either +/+ nu, +/0 nu or 0/0 nu embryos was used as reference standards for the nucleic acid hybridisation studies reported here.

Figure 2a gives the results of saturation hybridisation experiments with purified 18S + 28S ribosomal RNA annealed to DNA immobilised on nitrocellulose filters in the low temperature-formamide hybridisation conditions of McConaughy *et*

*al.*³². Detailed hybridisation information is given in the legend to Fig. 2. The hybridisation conditions chosen (2×SSC, 50% formamide, 45 °C) correspond to incubation at 25 °C below the *T_m* of *Xenopus* rRNA-DNA hybrids and ensures both a high specificity of annealing and fast reaction rate³³.

The specificity of the hybridisation reactions reported on here are indicated by the following findings: (1) there is only a slight reduction in the number of RNA-DNA hybrids retained on a filter after RNase treatment to eliminate partially paired hybrids³⁴; (2) reproducible saturation plateaux are attained after ribonuclease treatment of the hybrids; (3) unlabelled ribosomal RNA competes effectively with isotopically labelled rRNA in annealing experiments (Fig. 2b); (4) there is high thermostability of both sheared whole DNA-rRNA hybrids and chromatin monomer DNA-rRNA hybrids (Fig. 3) with *T_m* values in agreement with those reported by others^{26,35,36} in the same salt conditions; and (5) the saturation plateaux obtained with hybridisation of 18S and 28S ribosomal RNAs to DNAs from +/+ nu, +/0 nu, and 0/0 nu animals are within the range of expectation^{27,30,33}.

Figure 2 shows the results from one hybridisation experiment in which all DNA filters were annealed with increasing concentrations of the same batch of ³²P-labelled RNA in identical hybridisation conditions. Figure 2a shows that undigested DNA isolated from wild-type (+/+ nu) embryonic tissue culture cells reaches a saturation plateau of about 0.056% of the DNA hybridised even at low concentrations of input ³²P-rRNA (that is, at about 1.5 µg per ml of RNA). On the other hand, DNA from 0/0 nu embryos homozygous for the anucleolate mutation does not hybridise above the level of heterologous *E. coli* DNA, confirming the absence of detectable ribosomal cistrons in these embryos. DNA from heterozygous +/0 nu embryos has an intermediate saturation level between these two at about 0.032% hybridised corresponding to about 550 copies of the 18S and 28S ribosomal cistrons per cell. As is evident, the saturation plateaux for monomer, dimer, trimer and tetramer (and larger) DNA fragments fall above this heterozygote level but do not reach the level of hybridisation of undigested wild-type DNA. In addition, the saturation plateaux for longer pieces of DNA (in dimers, trimers and so on) are seen to reach increasingly higher levels compared with that of the monomer DNA. The plateau level for 18S+28S rRNA annealed to monomer DNA from exponentially growing tissue culture cells averages about 0.043% of the DNA hybridised (from four experiments). This represents about 72% of the level of hybridisation for bulk undigested DNA from the same cells. A similar reduction in the number of ribosomal cistrons in monomer DNA is obtained if hybridisation experiments are done with purified 28S ribosomal RNA alone (Table 1). Again, only about 73% of the number of 28S ribosomal genes present in sheared whole DNA are found in monomer DNA. These results most probably indicate that 28S and 18S ribosomal DNAs are both equally accessible to nuclease digestion in these transcriptionally active tissue culture cells.

As Fig. 2a shows, larger pieces of nuclease-cleaved DNA seem to contain greater numbers of rDNA fragments than do smaller pieces. Exact quantitation of the relative numbers of cistrons present in these increasingly long oligomers is difficult,

Table 1 Saturation hybridisation of DNA from logarithmic phase embryonic tissue culture cells with purified 28S and 5S ³²P-RNA

RNA*	DNA	µg of RNA per µg of DNA × 100 ± s.d. §	Mean no. of genes† copies per genome	No. of genes‡ as % of wild type
28S	Undigested	0.041 ± 0.002	982 ± 48	100
28S	Monomer	0.030 ± 0.003	718 ± 72	73
5S	Undigested	0.055 ± 0.005	56,485 ± 5,130	100
5S	Monomer	0.040 ± 0.008	45,170 ± 8,200	80

*Specific activity of ³²P-28S RNA was 6.4 × 10⁵ c.p.m. per µg RNA; of ³²P-5S RNA, 1.1 × 10⁶ c.p.m. per µg RNA.

†Calculations based on assumption that each somatic diploid cell contains about 6 pg of DNA^{26,29} and that 28S RNA has a molecular weight of 1.5 × 10⁶ and 5S RNA a molecular weight of 35,000 (refs 26, 27, 35 and 36).

‡The assumption is made that a weight of monomer DNA equivalent to the weight of DNA in a normal somatic cell is a genome equivalent.

§Standard deviations based on four experiments.

Table 2 Saturation hybridisation of adult erythrocyte DNA with ^{32}P -(28S+18S) RNA and ^{32}P -5S RNA

RNA*	DNA	μg of RNA per μg of DNA $\times 100 \pm \text{s.d.}^\dagger$	No. of genes ‡ as % of wild type
28S+18S	Undigested	0.058 ± 0.002	100
28S+18S	Monomer	0.050 ± 0.004	86
5S	Undigested	0.052 ± 0.002	100
5S	Monomer	0.046 ± 0.004	89

*Specific activity of the 28S+18S ^{32}P -RNA was 8.8×10^5 c.p.m. per μg RNA; of the ^{32}P -5S RNA, 1.2×10^6 c.p.m. per μg RNA.

† Assumptions as in Table 1.

‡ Standard deviations based on either three or four experiments in each case.

however, because of the limitations of sensitivity of nitro-cellulose filter hybridisation reactions.

Hybridisation using transcriptionally inactive cells

The mature nucleated erythrocytes of *X. laevis* are, as are most vertebrate erythrocytes, transcriptionally quiescent and do not synthesise detectable amounts of rRNA³⁷.

Chromatin from adult red blood cells was thus chosen for comparison with the transcriptionally active culture cells to determine whether there are detectable differences, in terms of accessibility to nuclease digestion, between synthetically active and inactive genes. The results of hybridisation experiments using 18S+28S rRNA or 5S RNA to saturate either bulk or monomer DNA from adult erythrocytes are given in Table 2.

The saturation levels for hybridisation of both types of RNA to undigested bulk erythrocyte DNA are similar to the plateaux for the same types of RNAs annealed to bulk culture cell DNA (Fig. 1 and Table 1). Erythrocyte monomer DNA (either obtained from gradients or eluted from gels after electrophoretic separation), however, consistently gave higher annealing plateaux for both the 18S+28S and 5S RNA populations than did monomer DNA derived from the synthetically active culture cells (compare Fig. 1 and Table 1). Thus an average of 86% of the 18S+28S ribosomal cistron DNA was protected from degradation in the erythrocyte chromatin subunits compared with about 72% being protected in the culture cell monomers. Similarly, close to 90% of the DNA coding for 5S RNA was recovered in blood cell monomers compared with only about 80% from embryonic cell subunits.

Structure of chromatin

The hybridisation results reported here support the notion that there is no simple, straightforward relationship between chromatin accessibility to micrococcal nuclease digestion and the transcriptional activity of the chromatin itself¹⁸⁻²¹. Most reiterated ribosomal cistrons and 5S genes are associated with protective proteins in the form of subunit particles whether the genes are being actively transcribed or not (Fig. 1, Tables 1 and 2). On the other hand, even in the chromatin of transcriptionally quiescent erythrocytes there are regions or stretches of DNA comprising up to 10-15% of the total reiterated gene copies that can be cleaved by nuclease attack (Table 2). Whether these 'open' regions of DNA in the inactive red cell chromatin reflect the fact that 10-15% of each of the multiple gene copies present are accessible to digestion or simply mean that 85-90% of all of the ribosomal genes are completely protected by monomer proteins cannot be determined from the data.

A similar ambiguity exists when considering the data presented in Fig. 1 and Table 1, showing that there are fewer ribosomal gene sequences protected from nuclease digestion in the chromatin of transcriptionally active cells than in chromatin that is not active in synthesis of rRNA. These data could be construed to mean that transcriptionally active DNA is more 'free' or 'open' than inactive DNA. But there are several technical and theoretical uncertainties, perhaps the foremost being the difficulty of interpreting hybridisation data concerning the transcriptional activity of genes that are present in multiple copies within a genome. For instance, it is not known

what fraction of the total number of reiterated genes is functional at any given time within a random population of growing cells. Even though RNA is synthesised continuously throughout the cell cycle (except in mitotic cells)³⁸, it is not at all clear that all of the genes coding for rRNA need be active in transcription at the same time.

An example of this latter problem for the somatic cells of *X. laevis* comes from the work of Knowland and Miller^{33,39}. These investigators have demonstrated that the absolute rate of ribosomal RNA synthesis is the same in wild-type (+/+ nu) and 0/+ nu embryos even though the latter embryos had only half the normal complement of ribosomal cistrons. These results were interpreted to mean that either the 0/+ nu embryos had doubled their rate of transcription of ribosomal genes or that the same number of active rRNA genes were present in both wild-type and mutant animals.

This report thus lends experimental support to a model of chromatin structure in which most of the DNA within genes is associated with proteins to form a 'chromatin subunit' whether or not the genes are actively involved in transcription. The data can also be interpreted to support the view that, during active transcription, genes may have all or some parts of their DNA in a more open or free configuration so as to allow ready access to heterologous molecules, such as experimentally applied nucleases. As discussed, however, this latter interpretation is still a matter of speculation. A direct test of this hypothesis might be to determine the nuclease accessibility of +/0 nu embryo chromatin (in which all of the ribosomal genes must be active for survival) before and after the onset of ribosomal RNA synthesis at the gastrula stage of development⁴⁰. Such experiments are in progress.

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Complete nucleotide sequence of bacteriophage MS2 RNA: primary and secondary structure of the replicase gene

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Bacteriophage MS2 RNA is 3,569 nucleotides long. The nucleotide sequence has been established for the third and last gene, which codes for the replicase protein. A secondary structure model has also been proposed. Biological properties, such as ribosome binding and codon interactions can now be discussed on a molecular basis. As the sequences for the other regions of this RNA have been published already, the complete, primary chemical structure of a viral genome has now been established.

RNA BACTERIOPHAGES such as MS2 have been important in molecular biology not only because they provide a model system for investigating viral RNA replication and the physiology of the infected cell, but also in the study of fundamental processes such as translation¹. MS2 RNA contains the genetic information to specify three viral polypeptides (Fig. 1). The first two genes code for the A protein and the coat protein, which are structural elements: the virus particle contains one A-protein molecule² and approximately 180 coat protein molecules. For practical reasons, most knowledge of the *in vitro* replication of phage RNA is based on work with a purified system derived from cells infected with the distantly related bacteriophage Q β (reviewed in ref. 3), but the principal findings are presumably also valid for the MS2 phage group⁴. The enzyme complex responsible for viral RNA replication has been referred to variously as viral RNA-dependent RNA polymerase⁵, RNA synthetase⁶ and replicase⁵. As the latter name is simple and unambiguous we use it here. The replicase complex consists of four polypeptides, α , β , γ and δ ^{7,8}. β is specified by the third and last viral gene and has a molecular weight of approximately 63,000 (ref. 4). α has been identified as the 30S ribosomal subunit S1 (ref. 9) and γ - δ as the elongation factors Tu-Ts^{1,3}.

The expression of the three viral genes is strictly regulated. The A-protein gene is presumably only translated from chains in a nascent state^{1,10,11}. The replicase gene is subject to a polar control by the coat gene; this means that the latter has to be translated to allow expression of the replicase gene^{1,10}. Moreover, the coat protein represses the replicase gene^{1,10} while the replicase complex in turn might be involved in shutting off coat gene expression¹². Regulation of the frequency of gene expression by modulation has also been proposed^{13,14}.

We have shown that the MS2 genome starts from the 5' end with a 129-nucleotide untranslated leader sequence (Fig. 1)¹⁵. Then follows the A-protein gene which starts with a G-U-G initiation codon and ends with a U-A-G codon^{11,16}, an intercistronic region of 26 nucleotides, the coat protein gene¹⁴, an intercistronic region of 36 nucleotides^{14,17}, the replicase gene (see below) and finally a 174-nucleotide untranslated segment at the 3' terminus^{16,18}. The proposed secondary structure models provide a rational basis for explaining such biologically relevant phenomena as the autocontrol of A-protein gene expression¹¹, the polarity effect¹⁴ and the location of easily mutable sites^{11,14}.

We now report the complete primary structure of the third and last MS2 gene, which codes for the replicase subunit. Some partial nucleotide sequences have been published before^{13,19,20}.

Nucleotide sequence and structure

Our methods, which have been described before^{11,16,18}, were briefly as follows. Uniformly ³²P-labelled MS2 RNA (2–8 mCi) was digested partially at 0 °C with the single-strand-specific ribonuclease T₁, and the digest was fractionated by electrophoresis on a polyacrylamide slab gel¹⁵. A series of bands was obtained, and the material in each band was separated further into individual fragments by two-dimensional gel electrophoresis²¹. The advantages of the latter method are that, in general, pure and unique fragments are obtained and that the procedure is applicable to virtually any chain length. These pure fragments were suitable for detailed sequence analysis, mainly according to the methodology described by Sanger and colleagues^{22,23}, or modifications thereof^{15,24}. The structural determination of the longer oligonucleotides required various methods^{25,26} and will be described in detail elsewhere.

Discrete fragments can be obtained reproducibly because of the specific three-dimensional conformation of the viral RNA; for example, a fragment may correspond to a hairpin. In milder conditions of partial enzymatic digestion, larger fragments are obtained, for example, several hairpins linked to each other or bound by secondary interactions. Although in this way several regions can often be ordered, this approach has practical limits. On the one hand, if a fragment becomes too large (for example, more than 300–400 nucleotides) its oligonucleotide composition can no longer be established accurately and on the other hand, some sites in the viral RNA are extremely sensitive to T₁ ribonuclease (see legend to Fig. 2). To solve this problem, we introduced the use of carboxymethylated ribonuclease (CM ribonuclease); this enzyme cleaves mainly between C and A

and between U and A residues²⁷. Such partial digestion of MS2 RNA with CM ribonuclease identified a complete new set of fragments. These not only often constituted suitable material for solving nucleotide regions not well represented in T₁ fragments, but by and large provided all the essential overlaps, which have enabled us to reconstruct the entire sequence.

For identification of the fragments and for correlating the T₁ fragments with the CM ribonuclease fragments, we used a catalogue of all the unique oligonucleotides (mainly T₁ oligonucleotides). In this respect, an improved minifingerprinting system²⁸ has been very helpful in the last stages of this work.

The nucleotide sequence of the replicase gene is shown in the form of a secondary structure model in Fig. 2. It starts with the initiation codon A-U-G and ends with the termination codon U-A-G; these regions have been published before^{14,16,18}. Figure 2 also shows sites which are sensitive to ribonuclease T₁, but which could be bridged by suitable CM-ribonuclease fragments, and vice versa for sensitive CM sites. For example, the T₁ fragment 2381-2532 was nearly always found in the same band of the primary gel separation system as the T₁ fragment 2533-2631, but no linkage in primary sequence could be demonstrated. Direct proof for the correct order, however, was provided by the characterisation of several CM fragments, such as 2500-2596.

Such fragments, clearly linked by secondary interactions and only separated in the denaturing conditions of the first dimension of the two-dimensional gel fractionation system²¹, provided the primary basis for the construction of the secondary structure model. Sometimes a unique pairing scheme could be derived, but more often there were several alternatives (usually varying only in detail). On the basis of simplified rules for estimating thermodynamic stability^{29,30}, the most plausible configuration can be selected. After these hairpins have been fixed, additional, thermodynamically stable interactions can then be sought. The model shown in Fig. 2 is much more tentative as far as the latter aspects are concerned. But it is satisfying that by and large the feathered arrows, which are a measure of the sensitivity of the site towards nucleases, point to loops at the top of hairpins, or to discontinuities in the helical segments or to other single-stranded loci. Further study of the model by chemical modification is in progress.

Some of the segments, which remained single stranded in the model shown in Fig. 2, can be base paired to other regions of the molecule (Fig. 3). Such long distance interactions (that is, linkage of segments far apart in the primary sequence) certainly agree with hydrodynamic and other physical properties of MS2 RNA^{31,32}, but they are not supported by direct experimental evidence. Some of these interactions may have important functional roles, as in the control of the genetic expression or in encapsidation. For example, the coat protein gene must be translated before the ribosome-binding region of the replicase gene becomes accessible. This polar effect can be explained by the interaction of segment 1409-1433 with segment 1738-1769 (Fig. 3), as proposed in a slightly different form¹⁴. This does not exclude that rearrangements to other conformations may occur^{14,33}.

Little can be said about the tertiary interactions of the type found in tRNA³⁴, which almost certainly are also present in MS2 RNA. Yet they may influence profoundly the structure-function relationship. Moreover, some alternative conformations which have not been retained in the present model (Fig. 2) may be stabilised by such tertiary interactions and thus may represent the true conformation.

Sequence of replicase subunit

On the basis of the nucleotide sequence, we can deduce the whole amino acid sequence of the replicase gene product (Fig. 4); only the first three amino acids had been established previously by direct peptide analysis^{35,36}. This is the first protein for which the primary structure has been solved entirely on the basis of the genetic information which encodes it.

The replicase subunit is a 544-amino acid polypeptide. Like

<i>a</i>					
	U	C	A	G	
U	Phe { 12 16 Leu { 8 ⑤	Ser { 7 12 6 10	Tyr { ⑤ 16 Ochre Amber 0	Cys { 5 2 Opal Trp 9	U C A G
C	Leu { 7 15 8 ⑦	Pro { 10 4 3 9	His { ④ ⑥ 7 8 Gln { 7 8	Arg { 11 13 ④ ⑧	U C A G
A	Ile { 7 13 ⑫ Met ⑨+9	Thr { 4 12 ⑧ 7	Asn { 11 7 9 16 Lys { 9 16	Ser { ④ 9 ④ ② Arg { ④ ②	U C A G
G	Val { 9 10 6 7	Ala { 15 7 8 12	Asp { 19 14 9 13 Glu { 19 14 9 13	Gly { 19 7 8 10	U C A G
<i>b</i>					
	U	C	A	G	
U	Phe { 19 29 Leu { 17 11	Ser { 15 20 16 22	Tyr { 9 32 Ochre 1 Amber 2	Cys { 6 6 Opal Trp 23	U C A G
C	Leu { 15 26 15 9	Pro { 17 10 9 13	His { 6 9 17 22 Gln { 17 22	Arg { 21 20 10 11	U C A G
A	Ile { 12 25 19 Met 2+18	Thr { 19 21 13 14	Asn { 17 28 19 26 Lys { 17 28 19 26	Ser { 8 16 7 6 Arg { 8 16 7 6	U C A G
G	Val { 21 21 16 1+18	Ala { 26 21 21 23	Asp { 28 22 16 28 Glu { 28 22 16 28	Gly { 37 16 12 16	U C A G

Fig. 1 The genome of MS2 contains three genes, shown in blocks. Untranslated regions are present at both ends of the viral RNA and between the genes. The length of the different regions, expressed in number of nucleotides, is shown on top. The nucleotides in the viral RNA are numbered from the 5' end to the 3' end and the positions of some important signals are indicated underneath (the initiation codon is considered part of the genes and the termination codon part of the untranslated region).

the A protein, it ends with an arginine residue. It is even more rich in arginine than the A protein (some arginine residues may be involved in interactions with the RNA). But as the aspartic acid content is also high (6.0%), the polypeptide is slightly less basic than the A protein. Also noteworthy are a relatively high leucine content (9.0%; also high in the A protein) and phenylalanine content (6.9%). On the other hand, valine, alanine and glutamine are low, compared with the two other viral proteins.

At present no very meaningful deductions can be made from the amino acid sequence. With available procedures it is not possible to derive a plausible conformation and even less to interpret the structure-function relationship. But when more

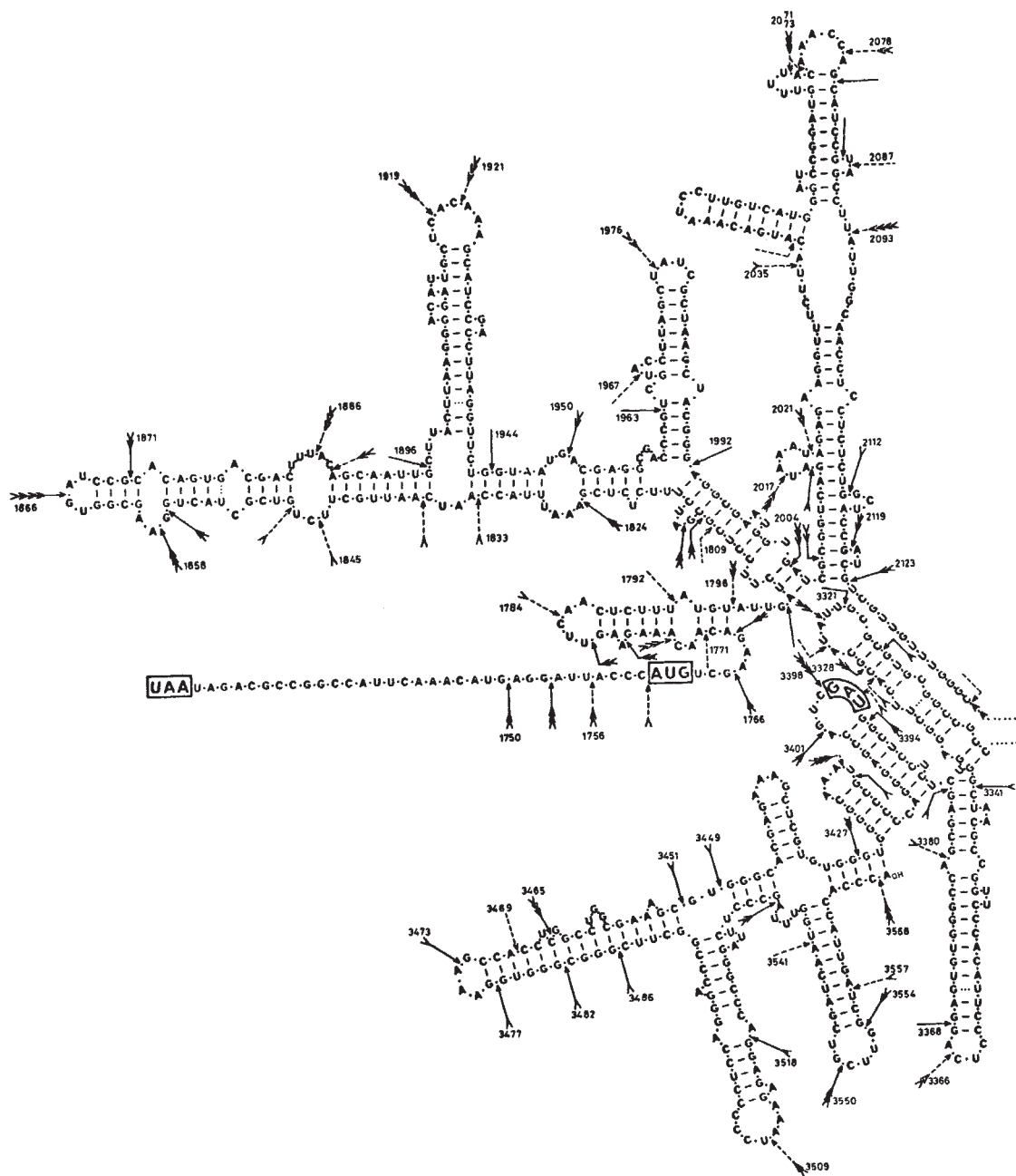
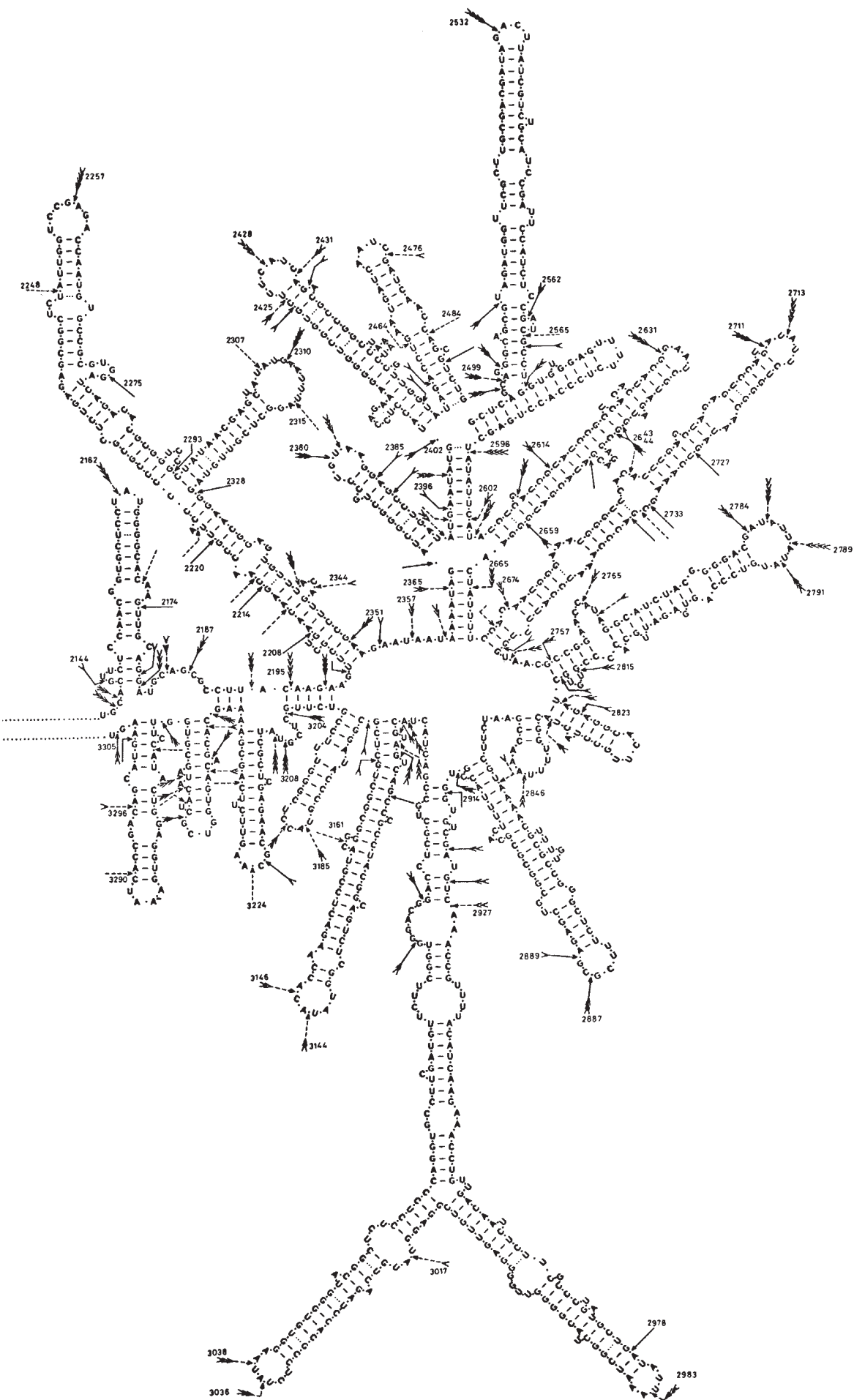


Fig. 2 Primary nucleotide sequence and secondary structure model of the replicase gene. The sequence is read from the 5' to the 3' end: U-A-A, termination signal of the preceding coat gene; an intergenic region of 33 nucleotides; A-U-G, the initiation codon of the replicase gene (1,632 nucleotides); U-A-G, the termination signal; an untranslated, 171-nucleotide 3'-terminal sequence. Together with previous models for the 5'-leader sequence-A-protein gene¹¹ and the coat gene¹⁴ this figure completes the chemical structure of the entire MS2 RNA. The numbering starts at the first (5') nucleotide and ends at the 3'-terminal residue 3569. The primary structure has been established rigorously but the secondary structure is based in part on experimental evidence and theoretical predictions and in part on conjectures (see text). Arrows point to sites easily split by nucleases during partial digestion of complete MS2 RNA: solid arrows, T₁ ribonuclease; dashed arrows, CM ribonuclease. The feathers indicate the sensitivity of the bond in the conditions used: (0) split very seldom, (1) seldom, (2) rather often, (3) very often, (4) always.



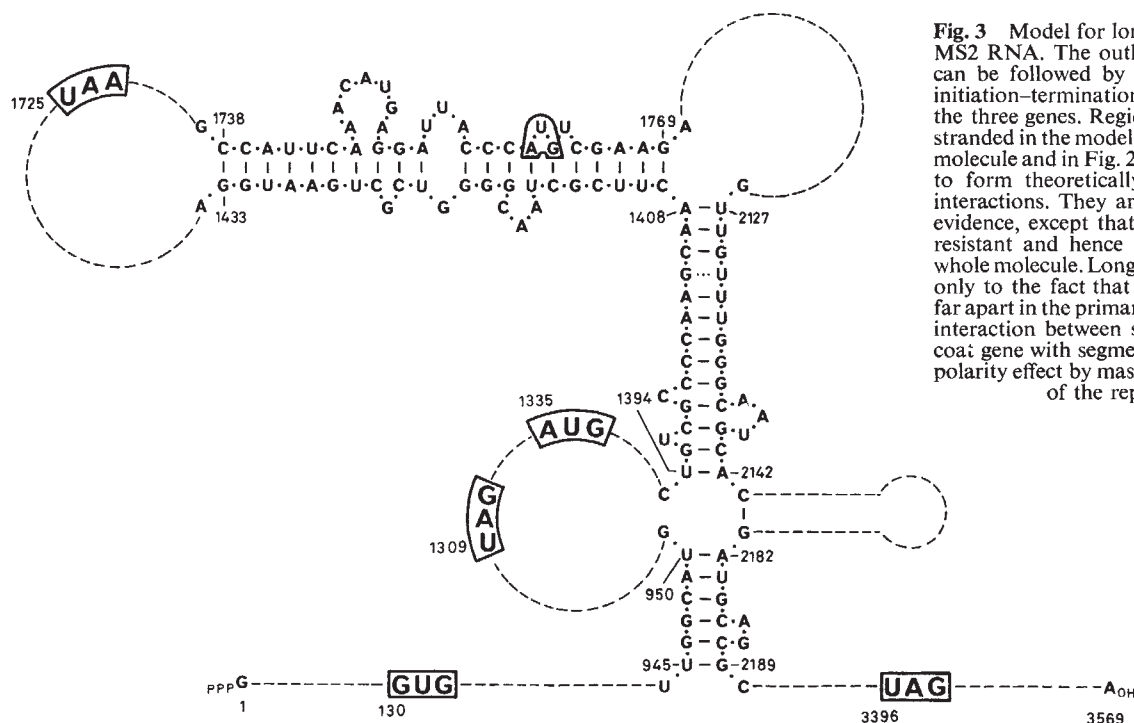


Fig. 3 Model for long distance interactions in MS2 RNA. The outline of the complete chain can be followed by the numbering and by the initiation-termination signals (in bold type) of the three genes. Regions which remained single stranded in the models for the other parts of the molecule and in Fig. 2, were screened for ability to form theoretically stable, complementary interactions. They are not supported by direct evidence, except that some are rather nuclease resistant and hence somehow protected in the whole molecule. Long distance interaction refers only to the fact that the segments involved are far apart in the primary sequence. Note that the interaction between segment 1409-1433 of the coat gene with segment 1738-1769 explains the polarity effect by masking the initiating A-U-G of the replicase gene.

Fig. 4 Nucleotide sequence of the replicase gene and amino acid sequence of the coded polypeptide. As in Figs 1-3, the nucleotides are numbered from the 5' end of the viral RNA. The amino acid sequence is entirely deduced from the nucleotide sequence data. In analogy with the Q β polypeptide it is assumed that the initiating formylmethionine has been eliminated in a post-translational process⁵⁵. This polypeptide, together with three host components S1, Tu and Ts, forms a complex which is responsible for specific viral RNA replication.

MS2 REPLICASE GENE

1764	A-U-G	U-C-G-A-A-G-A-C-A-A-C-A-A-A-G-A-A-G-U-U-C-A-A-C-U-C-U-U-U-A-U-G-U-A-U-U-G-A-U-C-U-U-C-C-U-C-G-C-G-A-U-C-U-U-U-C-U-C-U-C.	Ser - Lys - Thr - Thr - Lys - Lys - Phe - Asn - Ser - Leu - Cys - Ile - Asp - Leu - Pro - Arg - Asp - Leu - Ser - Leu - 20
1824		G-A-A-A-U-U-U-A-C-C-A-A-U-C-A-A-U-U-G-C-U-U-C-U-G-U-C-G-C-U-A-C-U-G-G-A-A-G-C-G-G-U-G-A-U-C-C-G-C-A-C-A-G-U-G-A-C-G-A-C.	Glu - Ile - Tyr - Gln - Ser - Ile - Ala - Ser - Val - Ala - Thr - Gly - Ser - Gly - Asp - Pro - His - Ser - Asp - Asp - 40
1884		U-U-U-A-C-A-G-C-A-A-U-U-G-C-U-U-A-C-U-U-A-A-G-G-G-A-C-G-A-A-U-U-G-C-U-C-A-C-A-A-A-G-C-A-U-C-C-G-A-C-C-U-U-A-G-G-U-U-C-U.	Phe - Thr - Ala - Ile - Ala - Tyr - Leu - Arg - Asp - Glu - Leu - Leu - Thr - Lys - His - Pro - Thr - Leu - Gly - Ser - 60
1944		G-G-U-A-A-U-G-A-C-G-A-G-G-C-G-A-C-C-C-G-U-C-G-U-A-C-C-U-U-A-G-C-U-A-U-C-G-C-U-A-A-G-C-U-A-C-G-G-G-A-G-G-C-G-A-A-U-G-G-U.	Gly - Asn - Asp - Glu - Ala - Thr - Arg - Arg - Thr - Leu - Ala - Ile - Ala - Lys - Leu - Arg - Glu - Ala - Asn - Gly - 80
2004		G-A-U-C-G-C-G-G-U-C-A-G-A-U-A-A-A-U-A-G-A-G-A-G-G-U-U-U-C-U-U-A-C-A-U-G-A-C-A-A-A-U-C-C-U-U-G-U-C-A-U-G-G-G-A-U-C-C-G.	Asp - Arg - Gly - Gln - Ile - Asn - Arg - Glu - Gly - Phe - Leu - His - Asp - Lys - Ser - Leu - Ser - Trp - Asp - Pro - 100
2064		G-A-U-G-U-U-U-U-A-C-A-A-A-C-C-A-G-C-A-U-C-C-G-U-A-G-C-C-U-U-A-U-U-G-G-C-A-A-C-C-U-C-C-U-C-U-C-U-G-G-C-U-A-C-C-G-A-U-C-G.	Asp - Val - Leu - Gln - Thr - Ser - Ile - Arg - Ser - Leu - Ile - Gly - Asn - Leu - Leu - Ser - Gly - Tyr - Arg - Ser - 120
2124		U-C-G-U-U-G-U-U-U-G-G-G-C-A-A-U-G-C-A-C-G-U-U-C-U-C-C-A-A-C-G-G-U-G-C-U-C-C-U-A-U-G-G-G-G-C-A-C-A-A-G-U-U-G-C-A-G-G-A-U.	Ser - Leu - Phe - Gly - Gln - Cys - Thr - Phe - Ser - Asn - Gly - Ala - Pro - Met - Gly - His - Lys - Leu - Gln - Asp - 140
2184		G-C-A-G-C-G-C-C-U-U-A-C-A-A-G-A-A-G-U-U-C-G-C-U-G-A-A-C-A-A-G-C-A-A-C-C-G-U-U-A-C-C-C-C-C-G-C-G-C-U-C-U-G-A-G-A-G-C-G.	Ala - Ala - Pro - Tyr - Lys - Lys - Phe - Ala - Glu - Gln - Ala - Thr - Val - Thr - Pro - Arg - Ala - Leu - Arg - Ala - 160
2244		G-C-U-C-U-A-U-U-G-U-U-C-C-G-A-G-A-C-C-A-A-U-G-U-G-C-C-G-C-G-U-G-G-A-U-C-A-G-A-C-A-C-G-C-G-U-C-C-G-C-U-A-U-A-A-C-G-A-G.	Ala - Leu - Leu - Val - Arg - Asp - Gln - Cys - Ala - Ala - Trp - Ile - Arg - His - Ala - Val - Arg - Tyr - Asn - Glu - 180
2304		U-C-A-U-A-U-G-A-A-U-U-U-A-G-G-C-U-C-G-U-U-G-U-A-G-G-G-A-A-C-G-G-A-G-U-U-U-A-C-A-G-U-U-C-C-G-A-A-G-A-A-U-A-A-A-A.	Ser - Tyr - Glu - Phe - Arg - Leu - Val - Val - Gly - Asn - Gly - Val - Phe - Thr - Val - Pro - Lys - Asn - Asn - Lys - 200

2364
A-U-A.G-A-U.C-G-G.G-C-U.G-C-C.U-G-U.A-A-G.G-A-G.C-C-U.G-A-U.A-U-G.A-A-U.A-U-G.U-A-C.C-U-C.C-A-G.A-A-A.G-G-G.G-U-C.G-G-U.
Ile - Asp - Arg - Ala - Ala - Cys - Lys - Glu - Pro - Asp - Met - Asn - Met - Tyr - Leu - Gln - Lys - Gly - Val - Gly - 220

2424
G-C-U.U-U-C.A-U-C.A-G-A.C-G-C.C-G-G.C-U-C.A-A-A.U-C-C.G-U-U.G-G-U.A-U-A.G-A-C.C-U-G.A-A-U.G-A-U.C-A-A.U-C-G.A-U-C.A-A-C.
Ala - Phe - Ile - Arg - Arg - Arg - Leu - Lys - Ser - Val - Gly - Ile - Asp - Leu - Asn - Asp - Gln - Ser - Ile - Asn - 240

2484
C-A-G.C-G-U.C-U-G.G-C-U.C-A-G.C-A-G.G-G-C.A-G-C.G-U-A.G-A-U.G-G-U.U-C-G.C-U-U.G-C-G.A-C-G.A-U-A.G-A-C.U-U-A.U-C-G.U-C-U.
Gln - Arg - Leu - Ala - Gln - Gln - Gly - Ser - Val - Asp - Gly - Ser - Leu - Ala - Thr - Ile - Asp - Leu - Ser - Ser - 260

2544
G-C-A.U-C-C.G-A-U.U-C-C.A-U-C.U-C-C.G-A-U.U-C-G.C-U-G.G-U-G.U-G-G.A-G-U.U-U-U.C-U-C.C-C-A.C-C-U.G-A-G.C-U-A.U-A-U.C-A.
Ala - Ser - Asp - Ser - Ile - Ser - Asp - Arg - Leu - Val - Trp - Ser - Phe - Leu - Pro - Pro - Glu - Leu - Tyr - Ser - 280

2604
U-A-U.C-U-C.G-A-U.C-G-U.A-U-C.C-G-C.U-C-A.C-A-C.U-A-C.G-G-A.A-U-C.G-U-A.G-A-U.G-G-C.G-A-G.A-C-G.A-U-A.C-G-A.U-G-G.G-A-A.
Tyr - Leu - Asp - Arg - Ile - Arg - Ser - His - Tyr - Gly - Ile - Val - Asp - Gly - Glu - Thr - Ile - Arg - Trp - Glu - 300

2664
C-U-A.U-U-U.U-C-C.A-C-A.A-U-G.G-G-A.A-A-U.G-G-G.U-U-C.A-C-A.U-U-U.G-A-G.C-U-A.G-A-G.U-C-C.A-U-G.A-U-A.U-U-C.U-G-G.G-C-A.
Leu - Phe - Ser - Thr - Met - Gly - Asn - Gly - Phe - Thr - Phe - Glu - Leu - Glu - Ser - Met - Ile - Phe - Trp - Ala - 320

2724
A-U-A.G-U-C.A-A-A.G-C-G.A-C-C.C-A-A.A-U-C.C-A-U.U-U-U.G-G-U.A-A-C.G-C-C.G-G-A.A-C-C.A-U-A.G-G-C.A-U-C.U-A-C.G-G-G.G-A-C.
Ile - Val - Lys - Ala - Thr - Gln - Ile - His - Phe - Gly - Asn - Ala - Gly - Thr - Ile - Gly - Ile - Tyr - Gly - Asp - 340

2784
G-A-U.A-U-U.A-U-A.U-G-U.C-C-C.A-G-U.G-A-G.A-U-U.G-C-A.C-C-C.C-G-U.G-U-G.C-U-A.G-A-G.G-C-A.C-U-U.G-C-C.U-A-C.U-A-C.G-G-U.
Asp - Ile - Ile - Cys - Pro - Ser - Glu - Ile - Ala - Pro - Arg - Val - Leu - Glu - Ala - Leu - Ala - Tyr - Tyr - Gly - 360

2844
U-U-U.A-A-A.C-C-G.A-A-U.C-U-U.C-G-U.A-A-A.A-C-G.U-U-C.G-U-G.U-C-C.G-G-G.C-U-C.U-U-U.C-G-C.G-A-G.A-G-C.U-G-C.G-G-C.G-C-G.
Phe - Lys - Pro - Asn - Leu - Arg - Lys - Thr - Phe - Val - Ser - Gly - Leu - Phe - Arg - Glu - Ser - Cys - Gly - Ala - 380

2904
C-A-C.U-U-U.U-A-C.C-G-U.G-G-U.G-U-C.G-A-U.G-U-C.A-A-A.C-C-G.U-U-U.U-A-C.A-U-C.A-A-G.A-A-A.C-C-U.G-U-U.G-A-C.A-A-U.C-U-C.
His - Phe - Tyr - Arg - Gly - Val - Asp - Val - Lys - Pro - Phe - Tyr - Ile - Lys - Lys - Pro - Val - Asp - Asn - Leu - 400

2964
U-U-C.G-C-C.C-U-G.A-U-G.C-U-G.A-U-A.U-U-A.A-A-U.C-G-G.C-U-A.C-G-G.G-G-U.U-G-G.G-G-A.G-U-U.G-U-C.G-G-A.G-G-U.A-U-G.U-C-A.
Phe - Ala - Leu - Met - Leu - Ile - Leu - Asn - Arg - Leu - Arg - Gly - Trp - Gly - Val - Val - Gly - Gly - Met - Ser - 420

3024
G-A-U.C-C-A.C-G-C.C-U-C.U-A-U.A-A-G.G-U-G.U-G-G.G-U-A.C-G-G.C-U-C.U-C-C.U-C-C.C-A-G.G-U-G.C-C-U.C-G.A-U-G.U-U-C.U-U-C.
Asp - Pro - Arg - Leu - Tyr - Lys - Val - Trp - Val - Arg - Leu - Ser - Ser - Gln - Val - Pro - Ser - Met - Phe - Phe - 440

3084
G-G-U.G-G-G.A-C-G.G-A-C.C-U-C.G-C-U.G-C-C.G-A-C.U-A-C.U-A-C.G-U-A.G-U-C.A-G-C.C-C-G.C-C-U.A-C-G.G-C-A.G-U-C.U-C-G.G-U-A.
Gly - Gly - Thr - Asp - Leu - Ala - Ala - Asp - Tyr - Tyr - Val - Val - Ser - Pro - Pro - Thr - Ala - Val - Ser - Val - 460

3144
U-A-C.A-C-C.A-A-G.A-C-U.C-C-G.U-A-C.G-G-G.C-G-G.C-U-G.C-U-C.G-C-G.G-A-U.A-C-C.C-G-U.A-C-C.U-C-G.G-G-U.U-U-C.C-G-U.C-U-U.
Tyr - Thr - Lys - Thr - Pro - Tyr - Gly - Arg - Leu - Leu - Ala - Asp - Thr - Arg - Thr - Ser - Gly - Phe - Arg - Leu - 480

3204
G-C-U.C-G-U.A-U-C.G-C-U.C-G-A.G-A-A.C-G-C.A-A-G.U-U-C.U-U-C.A-G-C.G-A-A.A-A-G.C-A-C.G-A-C.A-G-U.G-G-U.C-G-C.U-A-C.A-U-A.
Ala - Arg - Ile - Ala - Arg - Glu - Arg - Lys - Phe - Phe - Ser - Glu - Lys - His - Asp - Ser - Gly - Arg - Tyr - Ile - 500

3264
G-C-G.U-G-G.U-U-C.C-A-U.A-C-U.G-G-A.G-G-U.G-A-A.A-U-C.A-C-C.G-A-C.A-G-C.A-U-G.A-A-G.U-C-C.G-C-C.G-G-C.G-U-G.C-G-C.G-U-U.
Ala - Trp - Phe - His - Thr - Gly - Gly - Glu - Ile - Thr - Asp - Ser - Met - Lys - Ser - Ala - Gly - Val - Arg - Val - 520

3324
A-U-A.C-G-C.A-C-U.U-C-G.G-A-G.U-G-G.C-U-A.A-C-G.C-C-G.G-U-U.C-C-C.A-C-A.U-U-C.C-C-U.C-A-G.G-A-G.U-G-U.G-G-G.C-C-A.G-C-G.
Ile - Arg - Thr - Ser - Glu - Trp - Leu - Thr - Pro - Val - Pro - Thr - Phe - Pro - Gln - Glu - Cys - Gly - Pro - Ala - 540

3384
A-G-C.U-C-U.C-C-U.C-G-G U-A-G
Ser - Ser - Pro - Arg
544

data become available on viral and other RNA polymerases these amino acid sequences may reveal important clues on virus evolution and/or their origin.

Biological information content

The ribosome-binding region, including the initiating A-U-G of the replicase gene, was first identified by Argetsinger-Steitz³⁷. Good yields were only obtained after partial unfolding of the viral RNA. It is still not clear which features constitute a ribosome-binding region, although progress has been made. Shine and Dalgarno³⁸ have proposed that a purine-rich sequence preceding the initiation codon is involved in an interaction with a complementary 3'-terminal segment of the ribosomal 16S RNA. Indeed, all genuine ribosome-binding sites characterised so far contain a sequence of three or more purine nucleotides complementary to the 3' end of 16S RNA. Also, experimental evidence has been obtained for the direct complementary interaction between the ribosome-binding region and the 3' end of the 16S rRNA³⁹. But this cannot be the whole answer, because many regions in the viral RNA fulfil these criteria, yet are not bound. For example, the segment 2381-2396 is . . . U-A-A-G-G-A-G-C-C-U-G-A-U-A-U-G . . .; according to the criteria mentioned it should interact about as strongly as the A-protein initiation region with both *Escherichia coli* and *Bacillus stearothermophilus* 16S rRNA³⁸. That this is not observed may mean that masking/accessibility by secondary and tertiary structure may be equally important. Such cryptic sites may be involved in the additional initiations observed after formaldehyde treatment⁴⁰, but these are very unnatural conditions. On the other hand, ribosome binding as tested by protection against nuclease may not be as specific as polypeptide chain initiation. In the original fingerprints of the protected regions of the *E. coli* ribosome there were additional, unexplained spots³⁷; on the basis of preliminary identification (J. A. Steitz, personal communication) it seems likely that the main contaminating sequence contained the region 1965-1986. Likewise, in the experiment with *B. stearothermophilus* ribosomes at 49 °C (plate IV in ref. 41) a major contaminant must have been 2926-2967. In neither case is it immediately obvious why this anomalous binding reaction occurred.

Translation of the replicase gene is repressed by the coat protein, and the region to which the coat binds has been characterised⁴². The coat protein may interact with an alternative hairpin structure (Fig. 6 of ref. 14, and ref. 33).

Starting from the initiating A-U-G the nucleotides are read in triplets. For many regions the correct reading frame could be derived independently from the fact that the two illegitimate reading frames were blocked by nonsense code words¹⁹. The latter occur with a frequency not higher than expected for a random distribution. Finally the reading ends with the terminating U-A-G, as reported before¹⁸. This assignment is confirmed by studies of specific suppression in an *in vitro* translation system⁴³. The replicase gene is followed by a 174-nucleotide untranslated, 3'-terminal segment (including U-A-G); this is shown in a more compact model in Fig. 2.

A heat stable host factor, HF (a hexamer of 72,000 daltons), is required in the Q β -replicase reaction, but only when Q β -RNA plus strand is the template³. HF binds to two regions in Q β RNA, one of which is close to the 3' end⁴⁴. Although it is doubtful that HF is involved in the replication of the RNA of the MS2-R17-f2 group⁴⁵, it can nevertheless specifically bind to a single region in these viral RNAs. Senear and Steitz⁴⁴ found that HF protects a small segment in R17 RNA against nuclease, which has the following nucleotide sequence: . . . A-A-G-A-A-U-A-A-U-A-A-A-A-U-A-G . . .; undoubtedly this corresponds to the residues 2352-2367 which are located in the middle of the replicase gene. Presumably this interaction has no functional significance; HF is known to have a high affinity for poly(A)⁴⁶, and not only is the protected sequence A rich, but the T₁-oligonucleotide A-A-U-A-A-U-A-A-A-A-U-A-Gp has peculiar and very exceptional properties (strongly sticking to cellulose acetate and DEAE paper).

Use of code words

The code words used in the replicase gene are summarised in Fig. 5. On what basis is the choice made between degenerate codons for a given amino acid? In some cases it may be a historical accident, like the choice between a U-C-X or an A-G-Py codon for serine. It is unlikely that there are no constraints on the third letters. Indeed, although the mutation rate is very high (as evidenced by, for example, forward and backward mutation rates), we have been able to work for at least 6 yr with an essentially unaltered sequence. An obvious constraint is that third letters may be involved in optimising the secondary and tertiary structure. Secondary structure can be maximised by a proper choice of third letters and by bringing complemen-

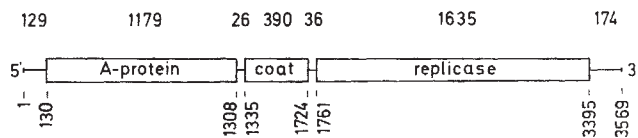


Fig. 5 Code words used in the replicase gene and in the whole genome. The numbers refer to the frequency with which each code word is used. *a*, Codewords used in the replicase gene. Circled numbers indicate codons not used in the coat gene. *b*, Summational table of codewords used in the three viral genes. The initiation codewords (one G-U-G and two A-U-Gs) are counted separately.

tary segments into proper register⁴⁷. Although some evidence in this direction was found^{13,14,48,49} in the case of the coat gene, it could not be substantiated further as more data became available¹¹. We believe that relatively few, selected third letters are sufficient to bring the molecule from a random secondary structure (50-60% base pairing^{49,50}; but this random structure is less stable⁵¹) to the level of a stable conformation as observed in the viral RNAs (73% $\pm$ 5 base pairing³¹). Statistical methods may not be adequate to reveal these effects convincingly. Moreover, we have no way of assessing the role of third letters in tertiary structure interactions. Neither should we forget that selection may also operate at the level of the negative strand (again no regions should be created which may function in ribosome interaction, or as nuclease targets or as binding sites for encapsidation and so on).

But it is very likely that at least for some amino acids other factors directly related to translation in *E. coli* have a predominant role in the choice of the third letter. For example, the code word G-G-U is definitely preferred for glycine and this is true for the whole genome (significant at the 0.1% level as tested by χ^2 analysis). This effect is specific and not due to a general preference for U in third positions, as found in the ϕ X174 gene G⁵².

We have suggested that isoleucine, tyrosine and/or arginine have modulating codons. Indeed, in the coat protein the codon U-A-U is not used for tyrosine, and we now conclude that for the whole genome U-A-C is definitely preferred (0.1% significance). Similarly, the code words C-G-Pu and A-G-Pu were not used for arginine in the coat gene, and it now seems that for the whole genome the preference is clearly C-G-Py > C-G-Pu > A-G-Pu (0.1% significance). These modulating triplets may be absolutely unacceptable in a gene like that for the coat, which is translated at high frequency, but can be tolerated in moderate numbers in genes whose elongation rate of translation is average. Isoleucine codons may constitute another class of modulating triplets. A-U-A is absent from the coat gene (5% significance; the coat contains only eight isoleucine residues), and is known to be recognised by a specific tRNA which is present in very small amounts in *E. coli*⁵³. The A protein and the replicase genes contain several A-U-A codons, and these may very well modulate (brake) the speed of translation; in fact, in these two genes it seems that it is the A-U-U codon which is selected against (1% significance).

In summary, we have established the primary nucleotide sequence of bacteriophage MS2 RNA. The polynucleotide chain contains 3,569 residues, 10.2% of which constitute untranslated segments. As all the genetic information is derived from this RNA molecule, the primary structure of all the virus-specified products (A protein, coat protein and replicase subunit) has also been deduced. MS2 is therefore the first living organism for which the entire primary chemical structure has been elucidated. We propose tentative models for the secondary folding of the viral RNA; parts of these are based on experimental evidence, and some aspects provide plausible bases for the explanation of biological effects. The secondary structure of the coat gene resembles a flower¹⁴, and there are similar foldings in other parts of the molecule; the secondary structure of the whole viral RNA therefore constitutes a bouquet.

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letters to nature

Solar influence on galactic cosmic ray anisotropy measurements

COSMIC ray particles of energies $\geq 10^{12}$ eV are virtually unaffected by the interplanetary magnetic field, and thus any galactic anisotropy manifests itself in sidereal intensity variation in Earth-based measurements. Below 10^{11} eV, on the other hand, solar modulation is of importance. Galactic anisotropy would be smeared out at this energy, and an anisotropy is introduced by the solar modulation effects. In the intermediate 10^{11} – 10^{12} eV region sidereal variation may originate either in genuine galactic anisotropy or in solar modulation¹. The London-Torino groups found², in accordance with other measurements³, that, in this intermediate energy range, the phase of the sidereal variation shifted from ~ 1800 to ~ 330 local sidereal time (LST) in 1969 when the polarity of the solar magnetic field reversed. The authors suggested that the change of phase was a result of the change of 'optics': different regions of the sky were scanned before and after 1969, respectively. Here we consider the alternative possibility—that the observed variation was a result of the electromagnetic field embedded in the solar wind.

At energies $> 10^{11}$ eV, the effect of magnetic irregularities is small and particle propagation is governed by the large scale interplanetary magnetic field. Thus, instead of using the transport equations which work well at lower energies, one has to consider individual particle trajectories. Anisotropy at the Earth will be introduced by the energy loss suffered by the particles in the solar system from the electric force associated with the expanding solar wind. If particles lose energy then,

at the Earth, we observe the galactic spectrum at higher energy, resulting in a lower flux. As in the London-Torino work², we take the magnetic field to be an Archimedean spiral directed away from the Sun everywhere north of the helioequator and towards the Sun south of it for the post-1969 epoch. The exact reverse field is assumed for the pre-1969 epoch. The electric field can be evaluated by requiring

$$0 = \mathbf{E} + \mathbf{V} \times \mathbf{B} \quad (1)$$

for particles comoving with the solar wind velocity $\mathbf{V}$. Thus we arrive at the following form of $\mathbf{B}$ and $\mathbf{E}$

$$\begin{aligned} B_r &= B_0(a/r)^2 & E_r &= 0 \\ B_\theta &= 0 & E_\theta &= B_0(a/r)(\Omega/c) \cos\theta \\ B_\phi &= B_0(a/r)(a\Omega/v) \cos\theta & E_\phi &= 0 \end{aligned} \quad (2)$$

r being the radial distance from the sun, $a = 1$ AU and B_0 being the radial component of the magnetic field at the Earth (2.5 gamma (ref. 2)). θ represents heliolatitude while Ω is the angular velocity of the rotating Sun ($\Omega = 2.7 \times 10^{-6} \text{ s}^{-1}$).

There are two implications of equation (2): (1) The electric field is independent of the solar wind velocity; (2) an electric quasi-potential can be introduced with

$$\mathbf{E} = -\nabla\Phi; \Phi = aB_0(a\Omega/c)|\sin\theta| \simeq 15|\sin\theta|(\text{MV}) \quad (3)$$

The potential, Φ , exists only within the solar cavity. If the Earth had a well defined potential then the energy loss would be identical for each trajectory, resulting in no anisotropy⁴.

In our model, however, the continuous injection of magnetic flux from the solar wind yields a $\nabla \times \mathbf{E}$ term, and it is this term which enables cosmic rays of different trajectories to reach the Earth having suffered different energy changes.

Particle trajectories depend only on rigidity, P ($P = pc/Ze$, p being the momentum and Ze being the electric charge of the particle). In the Solar System a highly relativistic particle changes its rigidity by

$$\Delta P = (dP/dE)\Delta E = -\Delta\Phi \quad (4)$$

where $\Delta\Phi$ is the potential difference between the Earth and the point where the particle entered the Solar System. Thus, for a fixed rigidity, P , the relative intensity variation follows as

$$\Delta I(P, t)/I(P) = -(\gamma + 2) \Delta\Phi(P, t)/P \quad (5)$$

where γ stands for the negative exponent of the differential rigidity spectrum of the galactic cosmic radiation. $\Delta\Phi(P, t)$ refers to the trajectory reaching the observation site vertically (most of the observed flux arrives nearly vertically). By taking the response function of the detector, $r(P)$, into account we obtain

$$\frac{\Delta I(t)}{I} = - \int_{P_0}^{\infty} (\gamma + 2) \frac{\partial r(P)}{\partial P} \frac{\Delta\Phi(P, t)}{P} dP$$

The detailed analysis of the trajectories carried out by the Imperial College group⁵ showed that particles of rigidities ≤ 300 GV were considerably deflected, while > 300 GV the deflection was small. As a rough approximation, we take that rigidities < 300 GV do not contribute to the intensity variation. Above 300 GV, on the other hand, trajectories are assumed

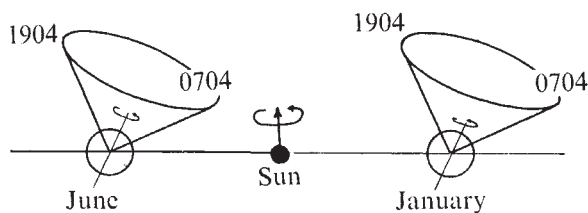


Fig. 1 The cone of the directions scanned for January and June. The maximal energy change occurs for the trajectory nearest to the pole, that is, for 1904 LST.

to be straight lines. Obviously the energy change is maximal for the trajectories nearest to the pole (Fig. 1) which results in a sidereal daily variation of 0.017% with times of maximum at 1904 and 0704 LST before and after 1969, respectively. This result is in accordance with the general character of the London-Torino measurement² ($0.013 \pm 0.003\%$; 1800 for the pre-1969 epoch and $0.012 \pm 0.005\%$; 0330 for the post-1969 epoch).

The model outlined here predicts opposite phases for measurements made on the Northern and Southern Hemispheres, respectively. In a measurement carried out at Hobart⁶ between 1957 and 1974 (the pre-1969 interval being dominant) the phase of the sidereal daily wave was found to be 0700 LST, agreeing with this prediction. There was, however, no sign of a reversal of phase in 1969. A detailed investigation of trajectories may give firmer support to the model discussed here or may disprove it. At present, however, one cannot rule out that the sidereal daily variation of cosmic ray intensity in the 10^{11} – 10^{12} eV region can be accounted for by solar influence.

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Ionising flux of cosmic background radiation

THE intensity of the diffuse cosmic background radiation for photon energies of 13.6 to ~ 150 eV cannot be measured directly because of absorption by the neutral component of the interstellar medium. In the range 150–300 eV, there is considerable galactic emission within a few hundred parsecs of the Sun which places severe limitations on attempts to measure the intensity and spectrum of the metagalactic background radiation at these energies. An indirect means of estimating the diffuse metagalactic flux of ionising radiation was proposed by Sunyaev¹, who studied the interaction of this radiation with neutral hydrogen at the peripheries of galaxies. More locally, the intensity of this radiation within ~ 1 kpc of the Sun can be probed by studying the ionisation of heavy elements in the interstellar medium². Here we re-examine Sunyaev's proposal in the context of more detailed studies of the interaction of metagalactic ionising radiation with galactic gas. We give a firm upper limit on the intensity of metagalactic ionising background radiation to which the neutral interstellar medium is exposed, and discuss some important consequences of this result. Our principal conclusion is that further than ~ 30 kpc from the centre of the Galaxy, self-shielding by H II^{3,4} is possible only if the flux of ionising radiation is below a certain critical value^{5–7}.

Hill⁶ used a self-gravitating galactic disk model of the galactic gas distribution that was exposed to an ionising photon flux calculated on a variety of possible extrapolations of the high latitude soft X-ray data to lower energies. Bochkarev and Sunyaev⁷ used a variety of possible gas density distributions, including disk models without stellar halos, and also models appropriate to a disk embedded in a massive non-gaseous corona.

An important result can be inferred from their studies: there is a critical value of the metagalactic ionising flux; if this value (Φ_c) is exceeded, there will be too little neutral hydrogen far from the centre of nearby spiral galaxies. The specific 21-cm observations that we use indicate that, after correcting for projection effects, the H I column density is $\sim (2-3) \times 10^{19} \text{ cm}^{-2}$ at ~ 30 kpc from the centre of M31 (refs 8–11). Now, the hydrogen ionisation rate from hydrogen-primary photo-ionisations at the periphery of the Galaxy is

$$\zeta = 4\pi \int_{\nu_H}^{\infty} \frac{\sigma\Phi}{h\nu} d\nu \simeq 4\pi\sigma_0\Phi_0 \left(\frac{\nu_H}{\nu_0}\right)^{3+\alpha} (3+\alpha)^{-1} h^{-1}$$

where we have written the incident ionising radiation flux ($\text{erg cm}^{-2} \text{ s}^{-1} \text{ Hz}^{-1}$) as $\Phi = \Phi_0(\nu_0/\nu)^\alpha$. Here ν_0 ($\nu \geq \nu_0 \geq \nu_H$) is a low energy cutoff, $\alpha \geq 0$, $\sigma_0 = 6.3 \times 10^{-18} \text{ cm}^2$, and ν_H is the frequency at the Lyman limit. It is important to also include the effects of helium recombination radiation and secondary electrons produced by both hydrogen and helium in the exact computations. This gives the critical value^{6,7} of ζ as $\zeta_c = 2 \times 10^{-13} \text{ s}^{-1}$, and this in turn gives an upper limit on the metagalactic ionising photon flux > 13.6 eV of $\Phi_c = 5 \times 10^{-23} (\nu_0/\nu_H)^\alpha \text{ erg (cm}^2 \text{ s ster Hz)}^{-1}$ if $\alpha = 0$ (and is readily generalised if $\alpha \neq 0$). ν_0 and α are characteristic parameters of the incident ionising flux: an unattenuated thermal bremsstrahlung spectrum with temperature parameter $kT \gg h\nu_H$ corresponds approximately to $\alpha = 0$ and $\nu = \nu_H$.

This limit depends weakly on the gas distribution; here it is for the model of a self-gravitating infinite disk with surface density varying inversely as galactocentric distance. For other models, somewhat lower limits are obtained that are within a factor of three of this value, which also lies in the same range as Sunyaev's earlier estimate¹.

These results were obtained under the assumption that the gas is diffuse. If there is an intercloud medium which is ionised and heated to $\sim 10^4$ K by the metagalactic ionising radiation, clouds cannot exist in thermally stable pressure equilibrium (and would consequently not be replenished over the cloud dissipation time scale) unless the mean gas density exceeds $\sim 0.03 \text{ cm}^{-3}$ (ref. 5). One remaining possibility would be for H I clouds at the periphery of the Galaxy to be maintained in pressure equilibrium with a tenuous, hot intercloud medium, for example at $T \simeq 10^6$ K and $n \simeq 10^{-3} \text{ cm}^{-3}$. The low column density of the clouds ($\sim 10^{19} \text{ cm}^{-2}$), however, renders them transparent to photons of energy $\gtrsim 50$ eV, and the clouds would evaporate over a time scale short compared with the relevant time scale for cloud formation, which we may identify with the cooling time of the ambient medium (particularly if the gas is depleted in heavy elements).

Note that the characteristic propagation time for the ionisation front in the diffuse medium is $\sim 1.7 \times 10^7 (\phi_c/\phi) (N/10^{20} \text{ cm}^{-2}) \text{ yr}$, where N is the local column density of gas normal to the galactic plane. If ϕ is sufficiently small, this can exceed the dynamical time scale in the outer regions of the Galaxy; in this case, it is necessary to study the propagation of a non-stationary ionisation front¹².

This conclusion has some notable implications that we now mention. Recent work on the existence of massive galactic coronae^{13,14} has indicated that such coronae may contain masses of $\gtrsim 10^{12} M_\odot$ within $\lesssim 100$ kpc of the Galaxy. Such coronae could be gaseous and unobservable if the gas temperature were in the range 10^5 – 10^6 K. Our limit on the ionising radiation flux, however, enables us to impose a severe restriction on the emission from a galactic corona containing gas in this temperature range, and we find that the maximum mass of a gaseous galactic corona (compare ref. 15) is

$$M_{\text{max}} \simeq 1 \times 10^{11} (\phi/\phi_c)^{1/2} (100 \text{ kpc}/R)^{5/2} (10^{-23} \text{ erg cm}^3 \text{ s}^{-1}/\Lambda)^{1/2} M_\odot$$

for a uniform density model, where R is the radius of the corona and $\Lambda(T)$ is the cooling function ($\sim 10^{-21}$ – $10^{-22} \text{ erg cm}^3 \text{ s}^{-1}$ for $T = 10^5$ – 10^6 K if the heavy element abundance, Z , is solar; $\Lambda \propto Z$). Consideration of a non-uniform gas distribution only strengthens this limit.

A second result is that we can give an upper limit on the mean luminosity, F , radiated by a QSO in the energy band 40–170 eV. Adopting Schmidt's¹⁶ QSO density distribution function and assuming a non-thermal continuum spectral energy distribution $\propto \nu^{-1}$ (ref. 17), we find from the background limit (in a $\Omega = 1$ cosmological model) that $F \lesssim 10^{29.2} \text{ erg s}^{-1} \text{ Hz}^{-1}$; this estimate takes into account the absorption of ultra-violet photons in the neighbourhood of the quasars. Since QSO optical luminosities (at 2,500 Å in the rest frame of the QSO) are in the range 10^{29} – $10^{31} \text{ erg s}^{-1} \text{ Hz}^{-1}$ (ref. 16) and several at high redshift are found to be transparent to Lyman continuum photons, we infer that QSO may be expected to make an appreciable contribution to the metagalactic ionising background radiation.

Finally we mention an upper limit on the mean density of intergalactic gas obtained by considering its radiation¹; the critical flux is exceeded only if the mean density of matter exceeds the value for closure of the Universe when a Hubble constant of $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$ is adopted (note that the radiation of the gas is proportional to H_0^3).

We realise that this discussion has hitherto been entirely devoted to the astronomy of upper limits. An important observational implication for 21-cm studies of external galaxies is that the presence of a metagalactic ionising background radiation field will affect the distribution of H I in the outer regions of

galaxies. The transition between H I and H II occurs over a dimension short compared with the galactocentric distance and with low spatial resolution should be detectable as an abrupt change in H I surface density (compare refs 4 and 5). Because of the hardness of the spectrum of ionising photons, however, the transition zone, in the case of a bremsstrahlung or power-law incident spectrum, can be appreciable, amounting to $\sim 2(\phi/\phi_0) (0.01 \text{ cm}^{-3}/n)^2 (T/10^4 \text{ K})^{1/2} \text{ kpc}$.

Thus the transition region may be detectable when high resolution 21-cm studies of the outlying parts of external galaxies are available, and would provide useful information on the nature of the ionising photon spectrum.

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New theory of the Great Red Spot from solitary waves in the Jovian atmosphere

THE nature and stability of the Great Red Spot (GRS) on Jupiter have been persistent problems. We show here that solitary waves on a horizontally sheared zonal flow in a rotating, stratified atmosphere can explain many of the GRS characteristics known at present and numerous other features that have been observed on Jupiter (see also ref. 1).

Solitary waves have a number of attractive properties: they are isolated, permanent waves in which nonlinear steepening balances dispersive, spreading effects². They can arise from arbitrary initial disturbances and interact nonlinearly without changing their shape². The only memory of an interaction is a finite spatial phase shift between the post- and the pre-interaction trajectories^{2,3}, an interaction which looks like a rapid acceleration of one wave through the other.

Their simplest mathematical basis is the quasi-geostrophic potential vorticity equation for an incompressible fluid⁴ which, when made suitably non-dimensional and when one assumes a flow composed of a mean zonal shear flow $U(y)$ and a superimposed disturbance $\varepsilon\psi(x, y, z, t)$, has the form

$$[\partial_t + U\partial_x + \varepsilon(\psi_x\partial_x - \psi_x\partial_y)] \times [\partial^2_{xx} + \partial^2_{yy} + (f_0/lNd)^2\partial^2_{zz}] \psi + (\beta - U'')\psi_x = 0 \quad (1)$$

The east-west, north-south and vertical coordinates are x, y, z ; t is the time; $f = f_0 + (U_0/l)\beta y$ is the local Coriolis parameter;

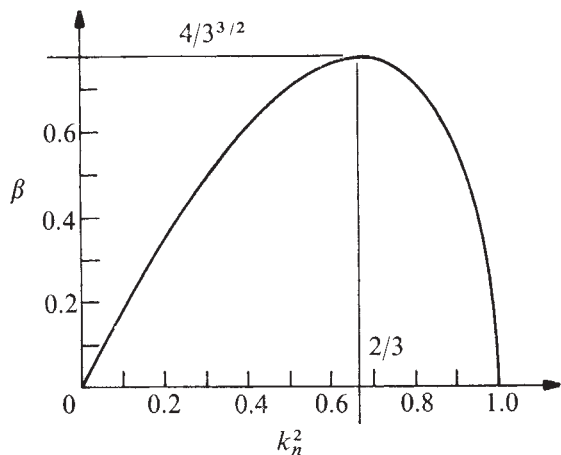


Fig. 1 Eigenvalue relationship for $U=\tanh(y)$; $\beta=2k_n^2(1-k_n^2)^{1/2}$ for $0 < k_n^2 < 1$.

N is the Brunt–Vaisala frequency (assumed constant) and $\mu=d/l$ the ratio of the atmospheric depth to the characteristic zonal wavelength.

Restricting our discussion to a single vertical wave mode, this equation admits a solution of the form

$$\psi = A(\xi, \tau) \phi(y) \cos(\pi \pi z) + O(\epsilon)$$

if $A(\xi, \tau)$ satisfies the modified Korteweg–DeVries equation

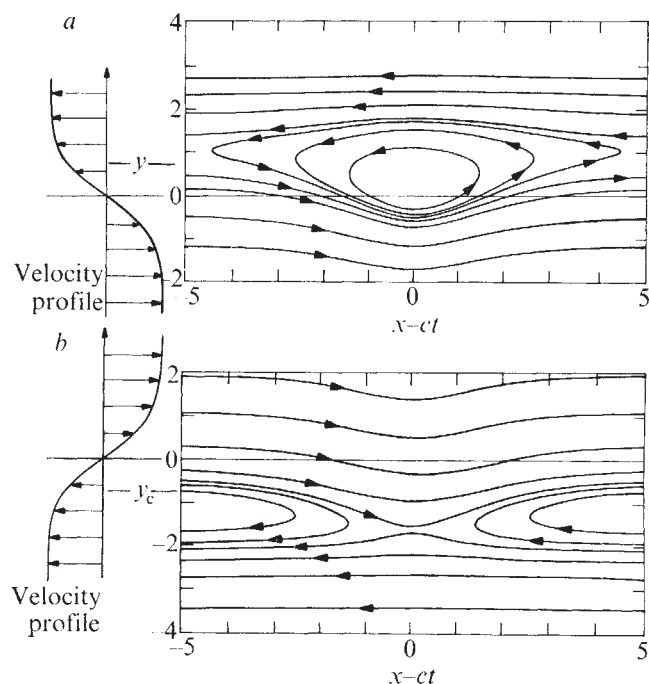
$$A_\tau + \alpha A^2 A_\xi + \gamma A \xi \xi \xi = 0 \quad (2)$$

where

$$\xi = \epsilon(x - c_0 t), \quad \tau = \epsilon^3 t$$

The quantities α and γ are numbers that depend on certain integral properties of ϕ , its derivatives and $U(y)$. Simultaneously, $\phi(y)$ satisfies the barotropic stability equation

Fig. 2 *a*, An E soliton on an anticyclonic shear in the southern hemisphere ($C=-0.7$, $\epsilon=0.7$). *b*, A D soliton on a cyclonic shear in the southern hemisphere ($C=-0.7$, $\epsilon=0.7$).



$$\begin{aligned} \phi'' - k_n^2 \phi + [(\beta - U'')/(U - c_0)] \phi &= 0 \\ k_n &= n\pi(f_0 l / Nd) \\ \phi &\rightarrow 0 \text{ as } |y| \rightarrow \infty \end{aligned} \quad (3)$$

For the shear profile, $U=\tanh(y)$, equation (3) has been solved by Lipps⁵. The appropriate eigenvalue curve for neutral solutions is shown in Fig. 1.

Figure 2*a* and *b* shows typical streamline patterns for waves corresponding to solitary waves of elevation (E solitons) and of depression (D solitons). The first has closed streamlines with pronounced cusps and the second has reversed flow fore and aft of the wave centre. The similarity of the flow shown in Fig. 2*a* to that found around the GRS⁶ is obvious and hardly needs further comment. The picture is enhanced when one constructs possible extensions of this theory to the solitary waves one would expect on the asymmetric jet-like profile that exists in the vicinity and equatorward of the GRS. Figure 3 shows a combined D–E soliton that emerges from a combining of flows like those shown in Fig. 2*a* and *b* with distortions arising from their mutual interactions. This picture is even closer to the total flow near the GRS, as shows particularly clearly in Fig. 4

There is strong evidence, both from its morphology and flow pattern (the so-called Circulating Current (Fig. 7, Plate III, Figs 5–9 of ref. 7)) that the South Tropical Disturbance (STD) was a D soliton. When this feature interacted with the GRS, observers noted a very rapid acceleration of the STD and its reformation without a change of shape (ref. 7, 137–143): we noted above that this is how solitary waves interact.

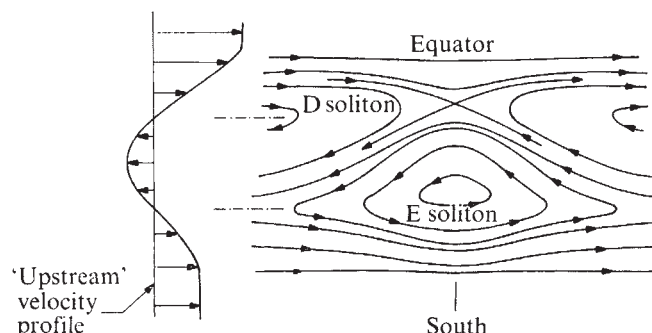


Fig. 3 A combined D–E soliton on an asymmetric jet-like profile.

There are a variety of similar examples. The Dark South Tropical Streak (ref. 7, plate IX) has the structure of a D soliton. Again many of the 'spot-like' features in ref. 7, plates V and X, and also in the Pioneer 10 and 11 pictures (the Little Red Spot, for example) sit in regions between cloud bands, where one would expect horizontal shear to exist and have the morphology of E solitons. The 'plume' at the northern edge of the equatorial jet, seen in the Pioneer pictures and the historical record⁸, seems to be one branch of a D soliton.

One can also extend, by inference, the present weakly nonlinear theory to a highly nonlinear regime. Then one would expect supercritical flows, with hydraulic jumps, or leewaves, to appear 'downstream' of the solitary waves (as in flow over bodies in rotating or stratified flows). Such flows have been observed around the GRS and can be seen in Fig. 4 downstream of the 'White Oval' below and to the left of the GRS.

The theory also places certain restrictions on the atmospheric parameters, and we now show that these are consistent with available models and observations. Taking a typical point on the eigenvalue curve, $k_n^2 \approx 0.8$, $\beta = 0.7$ with $U_0 \approx 55 \text{ m s}^{-1}$ (ref 6), and a latitude of 24° , we obtain $l \approx 3,000 \text{ km}$ (corresponding to a shear zone width for the $\tanh(y)$ profile of $9,000 \text{ km}$) and $Nd/n \approx 1,500 \text{ m s}^{-1}$. For example, an isothermal stratosphere at 110 K has $N=2.2 \times 10^{-2} \text{ s}^{-1}$, in which case $d/n \approx 6.7 \text{ km}$, or four density scale heights. For more realistic atmospheric models in which N increases from zero, at a depth near the

ammonia cloud base, to a maximum at an inversion, our restriction on Nd/n becomes, crudely, one on

$$\int_0^d (N/n) dz$$

and more appropriately, we interpret the resulting 'averaged' value of d/n as the vertical wavelength of the wave. In this case

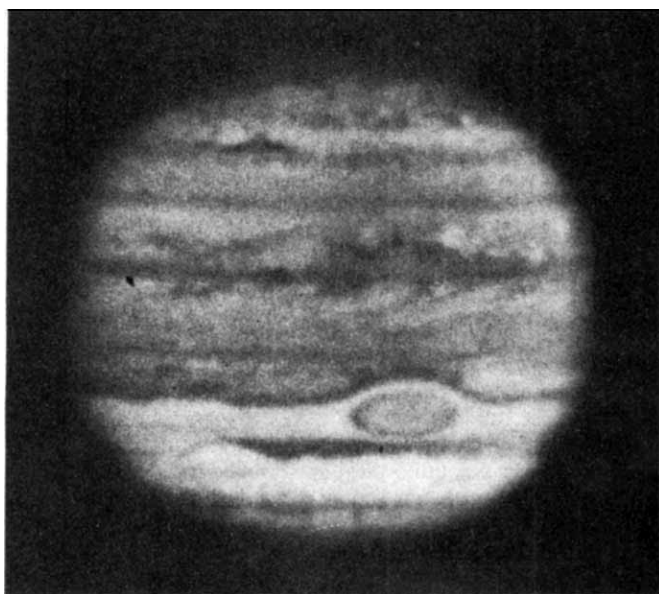


Fig. 4 Photograph taken at Pic-du-Midi Observatory on November 23, 1964 through a yellow filter (photo courtesy of Lowell Observatory) together with an interpretive sketch of the flow field that is consistent with the observations of Reese and Smith⁶. Compared to Fig. 3, this suggests that the GRS and Hollow can be interpreted as a combined D-E soliton. Also note the leewave type of flow downstream of the White Oval below and to the left of the GRS.

the wave now propagates within the cloud layer to a depth at which N is effectively zero (decaying exponentially below this level) and to a height determined by the constraint on

$$\int_0^d (N/n) dz$$

For the model of Wallace *et al.*⁹, most of the first vertical amplitude oscillation will be in and below the inversion level. Thus, in this model the GRS is not a deep-seated feature, but one that propagates probably no deeper than one scale height below the base of the ammonia cloud layer. In this sense, it differs from the available explanations of Hide¹⁰, Ingersoll¹¹, Smoluchowski¹² and Kuiper¹³, which place most of the motion below the tops of these clouds. Finally, we note that since the flow is related to the corresponding instability of the barotropic shear flow that makes up the basic state, the wave is able to

extract energy from this flow and maintain itself in the presence of dissipation, possibly accounting for the long lived nature of the GRS.

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Preferential adsorption of ^{137}Cs to micaceous minerals in contaminated freshwater sediment

A MAJOR concern to environmentalists, health physicists and radioecologists is the fate of ^{137}Cs released to the environment from existing light-water power stations (estimated annual discharge of ^{137}Cs as liquid effluent exceeds 2.0 Ci per yr per power station) and future nuclear fuel reprocessing facilities¹. Its long half life (30 yr) and its ability to be readily assimilated into body tissues make it potentially one of the most hazardous radionuclides for man. Numerous investigations into the fate of ^{137}Cs in terrestrial environments have revealed that soils exhibit a marked capacity to retain ^{137}Cs ; thus soils act as a 'sink' for ^{137}Cs released to the environment^{2,3}, and sediments retain ^{137}Cs in a similar fashion in aquatic environments^{4,5}.

We report here the detection of preferential adsorption of ^{137}Cs to the micaceous component of a ^{137}Cs contaminated freshwater sediment, containing predominately kaolinite and quartz, using density-gradient mineral segregation in a large scale zonal rotor¹⁰.

Experiments on reference clay minerals have shown that the single most important factor controlling caesium retention is the ability of certain layer silicates such as micas, vermiculites and interleaved micas (formerly called illites) to preferentially adsorb or 'fix' trace quantities of Cs (ref. 6). The 'fixation' mechanisms of these minerals are a result of the trapping of Cs ions in either the interlayer regions of vermiculite, a partially expanded mineral, or at the frayed edges of illites and micas⁷. ^{137}Cs is more strongly retained in soils containing predominately micaceous minerals, thus resulting in considerably less uptake of ^{137}Cs in plants grown on these soils as compared with tropical soils⁸, peat soils⁸ and acid sandy podzolic soils⁹, which do not contain large quantities of micaceous minerals. In no instance before has preferential adsorption of ^{137}Cs to the micaceous component of a soil or sediment containing a variety of minerals been demonstrated in environmental conditions.

Sediment particles from Steel Creek (obtained in a previous experiment¹¹) were largely distributed in three ranges: a large band covering the density range from 2.21 to 2.47 g cm⁻³, a smaller band between 2.47 and 2.57 g cm⁻³ and a band, mostly of quartz, from 2.57 to 2.65 g cm⁻³ (Fig. 1). These bands constituted approximately 48, 22 and 17% of the recovered sediment,

Table 1 Description of the major density ranges of sediment particles fractionated by density-gradient centrifugation in a large scale zonal rotor

Sediment particle density range (g cm ⁻³)	Mineral composition* (%)	Fraction by		¹³⁷ Cs enrichment factor†
		Weight	¹³⁷ Cs activity (%)	
<2.21	95 Kl	7.1	5.1	0.72
2.21–2.47	95 Kl, 5 Qz	48.4	60.8	1.26
2.47–2.57	80 Kl, 15 Qz, 5 Mi	21.7	16.1	0.74
2.57–2.65	90 Qz, 5 Kl, 5 Mi	16.8	8.7	0.52
2.65–2.76	85 Mi, 15 Kl	1.9	6.0	3.16
>2.76	Hm	4.0	3.3	0.82

*Kl = Kaolinite, Qz = quartz, Mi = includes interleaved micas and muscovite, Hm = heavy minerals fraction containing anatase, iron-rich chlorite, traces of mica, and iron-rich sesquioxides. The quantities of each mineral were estimated based on their respective X-ray diffraction peak heights.

† Ratio of the fraction of ¹³⁷Cs activity to the weight fraction.

respectively (Table 1). Before density separation, X-ray diffraction showed only the minerals kaolinite and quartz in the <50-μm fraction (Fig. 2a). After fractionation, X-ray diffraction analyses revealed the mineral component in the density range 2.21–2.47 g cm⁻³ to be ~ 95% kaolinite (Fig. 2b), whereas the density range between 2.47 and 2.57 g cm⁻³ contained ~ 80% kaolinite and some quartz (~ 15%) and a little muscovite mica (~ 5%). The third major band (density range 2.57–2.65 g cm⁻³) contained approximately 90% quartz, 5% kaolinite and 5% muscovite mica (Fig. 2c). The ¹³⁷Cs concentrations in these bands are ~ 1.0, 0.6 and 0.4 nCi g⁻¹, respectively. We attribute the decreasing ¹³⁷Cs concentration with increasing particle density to the increasing concentration of quartz (Table 1).

The highest ¹³⁷Cs concentration was observed in the particle density range from 2.65 to 2.76 g cm⁻³ (Fig. 1). The ¹³⁷Cs concentration in this density fraction (maximum > 3.0 nCi g⁻¹) was three times that observed in the density fraction 2.21–2.47 g cm⁻³, whose major mineral was ~ 95% kaolinite. The mineral component in the 2.65–2.76 g cm⁻³ density fraction was ~ 85% muscovite (Fig. 2d). The quantity of sediment particles isolated in this density range (2.65–2.76 g cm⁻³) was only 0.28 g or <2% of the recovered sediment sample. Thus, through the use of density-gradient centrifugation in a large scale zonal rotor, sufficient quantities of the muscovite mica component of the sediment could be separated from the dominant minerals, kaolinite and quartz, so that the ¹³⁷Cs

concentration associated with the micaceous component could be determined. This is the first explicit demonstration that ¹³⁷Cs is selectively adsorbed to 'Cs-fixing' minerals in environmental conditions.

Approximately 6% of the total ¹³⁷Cs burden is directly associated with muscovite and >80% is associated with density fractions whose major mineral component is kaolinite (Table 1). Less than 20% of the ¹³⁷Cs associated with the dominant kaolinite band (2.27–2.33 g cm⁻³) could be displaced with 2 N CsCl and only 22% could be displaced with 5 N NaCl. On the other hand, 75–83% of the ¹³⁷Cs could be displaced from reference kaolinite clay using 1 N NaOAc⁴, indicating minerals other than kaolinite are responsible for the observed 'fixation'. Delineating the ¹³⁷Cs 'fixation' mechanism for these kaolinite bands is beyond the scope of this paper. It is conceivable, however, that the quantities of interleaved micas present were sufficient to 'fix' ¹³⁷Cs, but could not be detected by X-ray diffraction procedures. Interleaved micas, because of their irregular interlayer spacing, are difficult to detect by X-ray diffraction. Soils that contain appreciable quantities of mica in the density range between 2.65 and 2.78 g cm⁻³ also contain interleaved micas that band between 2.21 and 2.47 g cm⁻³. Mineral segregation in density-gradient centrifugation is good, but not perfect because of the incomplete dispersion of minerals in the non-aqueous density gradient¹⁰. Sufficient quantities of either interleaved micas or muscovite micas may have been present in the light density kaolinite band to provide

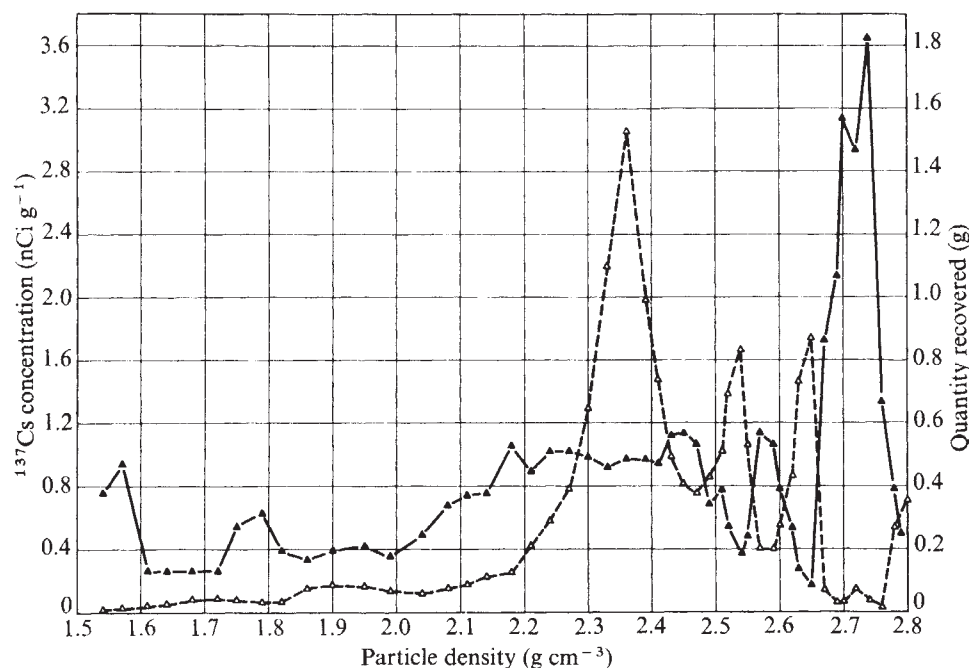
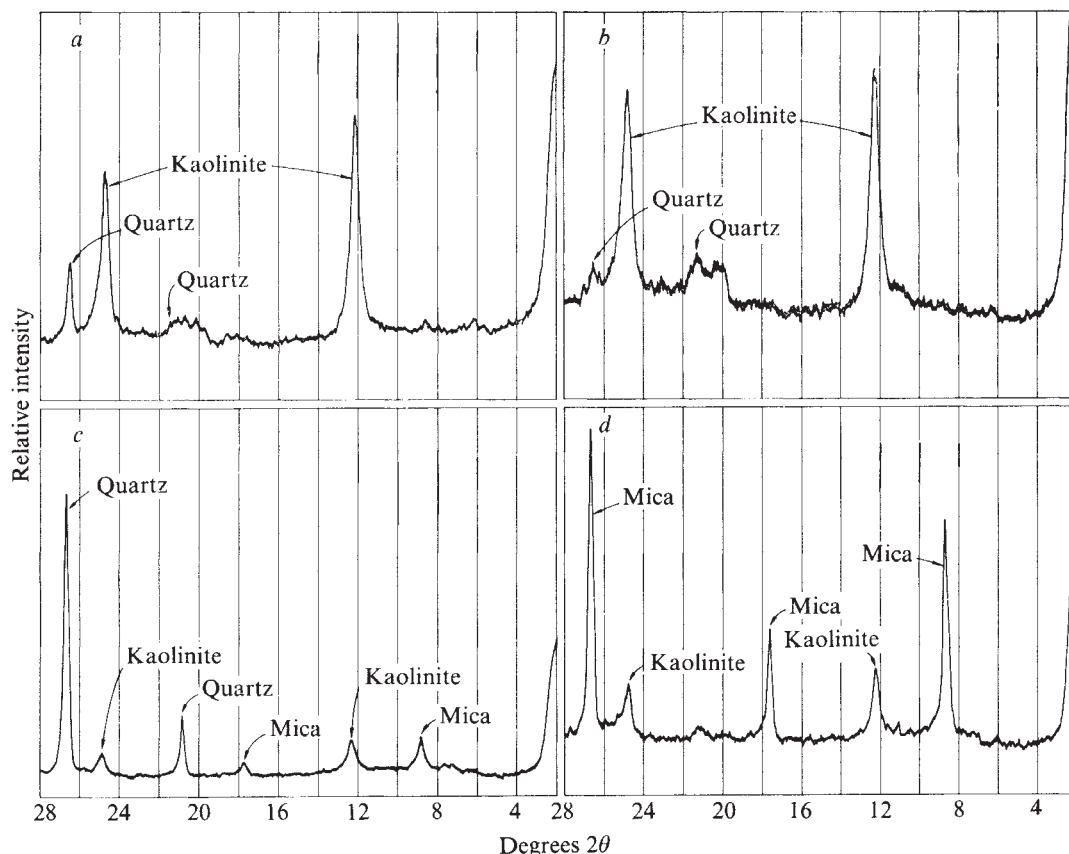


Fig. 1 Distribution of ¹³⁷Cs in sediment from Steel Creek, South Carolina. The concentration of ¹³⁷Cs in nCi g⁻¹ (▲) and quantity of sediment recovered in g (△) at various densities (g cm⁻³) after density-gradient centrifugation in a large scale zonal rotor.

Fig. 2 X-ray diffraction patterns of the basal (001) diffraction maxima from oriented aggregate mounts of *a*, Steel Creek sediment, <50 μm diameter; *b*, particle density fraction at 2.36 g cm^{-3} ; *c*, particle density fraction at 2.65 g cm^{-3} ; and *d*, particle density fraction at 2.74 g cm^{-3} . The abscissa units are degrees 2θ of Bragg's law and the ordinate reflects the relative intensities of the diffraction maxima using a Philips X-ray diffraction unit with a copper tube and nickel filter



'fixation' sites. For example, the ^{137}Cs associated with the density band identified as predominately a kaolinite mineral, is adsorbed to the weathered and unweathered mica minerals which cannot be detected by conventional X-ray diffraction procedures. Detailed examinations of a Georgia kaolinite using high resolution electron microscopy of ultramicrotomed sections of the kaolinite mineral revealed not only the expected 7 Å (001) spacing, but also occasional 10 and 14 Å spacings¹². These higher interlayer spacings are thought to represent occluded and interleaved micas which will fix ^{137}Cs .

The consequence of selective adsorption of Cs to 'Cs-fixing' minerals in environmental conditions strongly influences the availability of ^{137}Cs cycling to the biotic component of a particular ecosystem. For example, as the ^{137}Cs diffuses from the less selective sites of soils or sediments to the more selective sites that 'fix' ^{137}Cs , the burden to biological organisms in that environment will be appreciably decreased. A case in point is the work of Eyman and Kitchings¹³ who demonstrated that uptake of ^{137}Cs in bluegill (*Lepomis macrochirus*) and channel catfish (*Ictalurus punctatus*) from ingested clays containing adsorbed ^{137}Cs was ~ 8 times greater from montmorillonite and kaolinite than the interleaved mica, illite.

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Oxygen isotopic evidence for the development of the psychrosphere 38 Myr ago

THREE major elements were involved in the evolution of oceanic circulation during the Cainozoic. The first of these was the diminishing role of oceanic meridional circulation in equatorial areas, the second was the development of circum-Antarctic circulation and the third involves the development of the present-day system of bottom waters of the world ocean, the 'psychrosphere'^{1,2}. Because deep bottom waters are mostly derived from the polar regions, their characteristics, such as temperature, reflect surface conditions at high latitudes³. Past temperature changes of deep oceanic waters, and thus of polar surface waters, can be determined from the oxygen isotopic composition of deep-dwelling benthonic microfossils^{3,4}. A deep-sea palaeo-temperature record has been established⁴ for the past 54 Myr based on DSDP sites in the sub-Antarctic; detailed arguments⁴ give evidence that the Antarctic ice sheet developed during the Middle Miocene. Previous to this, however, a significant 5 °C decline in bottom-water temperature is recorded close to the Eocene-Oligocene boundary and is considered⁴ to represent the onset of Antarctic bottom-water formation at temperatures close to freezing. This heralds the beginning of the modern thermo-haline circulation and is thus one of the most important palaeo-oceanographic events in the Cainozoic. We examine here

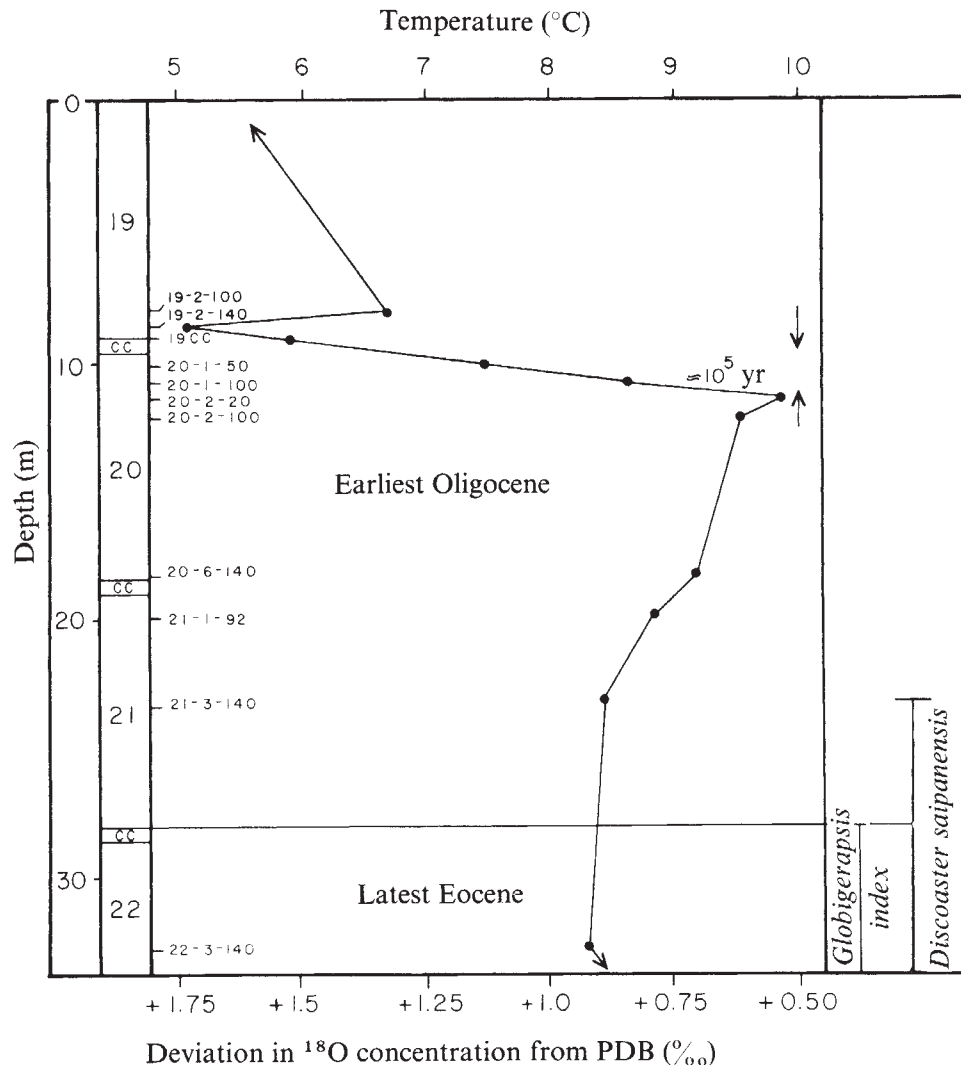


Fig. 1 Palaeotemperature curve and oxygen isotope data for benthonic foraminifera in latest Eocene-earliest Oligocene sediments of DSDP Site 277 (sub-Antarctic). Bottom temperature values were calculated using the procedure of Shackleton and Kennett⁴. The duration of the temperature drop of $\sim 4^\circ\text{C}$ within core 20 is calculated from sedimentation rates at between only 75,000 and 100,000 yr. The Eocene-Oligocene boundary in this site is marked by the extinction of *Globigerapsis index*⁶ as in New Zealand³². Thickness is plotted from top of core 19 which is 168.5 m below the ocean floor. Sample levels are shown.

in more detail the palaeotemperature event near the Eocene-Oligocene boundary, the latter having an age of 38 Myr (ref. 5).

Oxygen isotope measurements have been made on a series of latest Eocene to earliest Oligocene samples from cores 19 to 22 in DSDP Site 277, located on the Campbell Plateau in present day sub-Antarctic waters south of New Zealand ($52^\circ 13.43'\text{S}$; $166^\circ 11.48'\text{E}$; 1,222 m). This sequence consists of continuously cored late Palaeocene to late Oligocene foraminiferal-rich calcareous sediments⁶. About 38 Myr ago the southern part of the Campbell Plateau was at a palaeolatitude of $\sim 65^\circ\text{S}$, 1,200 km nearer to Antarctica than now⁷. Samples of mixed benthonic foraminifera were analysed in 11 samples which consisted of ~ 0.1 and 0.4 mg carbonate after cleaning. Analytical methods, calibration and reproducibility of results have been discussed elsewhere⁴⁻⁸. The Antarctic continent was essentially ice-free before the Middle Miocene⁴, and isotopic changes near the Eocene-Oligocene boundary are considered to have resulted from temperature changes.

A rapid reduction in bottom-water temperature from 10°C to 5°C during the earliest Oligocene is shown from the upper part of core 20 to the lower part of core 19 (Fig. 1), immediately followed by a brief warm oscillation (sample 19-2-100). Over half of the rapid temperature drop is represented in the uppermost part of core 20, the remainder between the upper sample of core 20 and the base of core 19 (sample 19-2-140). The sequence in core 20 represents a total recovery of 9 m (ref. 6) and thus the stratigraphic position of individual samples within this core is well established. The exact stratigraphic position of

samples in core 19 relative to core 20 is unknown because of incomplete core recovery. Although there are now no geochronological methods to date the sediment of this apparently continuous sequence directly, the speed of the palaeotemperature change can be estimated using sedimentation rates. The rapid palaeotemperature change (Fig. 1) occurs over a thickness of only 170 cm. Sedimentation rates of ~ 1.6 – 2.2 cm per 1,000 yr for this interval⁶ show that the duration of the palaeotemperature change was in the order of only 75,000–100,000 yr. About half (2.4°C) of the temperature change recorded in the unbroken sequence at the top of core 20, took place in a period of 55,000–75,000 yr. The change occurs within the earliest Oligocene-latest Eocene *Globigerina brevis* foraminiferal zone⁹ and the lower part of the *Reticulofenestra oamaruensis* to the top of the *R. placomorpha* calcareous nannofossil zones¹⁰. As in New Zealand, the Eocene-Oligocene boundary is placed at the extinction of *Globigerapsis index* (Fig. 1), and thus the temperature change occurs within the earliest Oligocene as recognised in temperate areas. At Site 277 the extinction of *Discoaster saipanensis* also precedes the isotopic event (Fig. 1). Further to the north in New Zealand, the extinction of *D. saipanensis* slightly precedes that of *G. index*¹⁰. A striking isotopic change in shallow-water benthonic fossils has also been shown close to the Eocene-Oligocene boundary in New Zealand sequences¹¹. In the tropics, a decrease in bottom-water temperatures of $\sim 5^\circ\text{C}$ is reported within the late Eocene between zones P15 and P17 (ref. 12), a change which must be isochronous with the sub-Antarctic event. This indicates that biostratigraphic correlations need modification between tropical, temperate and sub-Antarctic sequences. The extinctions of critical species

probably occurred earlier in high latitude sequences creating diachronous usage of the Eocene-Oligocene boundary.

Our interpretation of the latest Eocene-earliest Oligocene oxygen isotopic change^{4,11-13} is that it reflects a decrease in Antarctic surface-water temperatures to freezing and the production of extensive sea ice. This also marks the development of the psychrosphere. The oxygen isotopic evidence⁴ indicates that no significant ice sheet formed at this time, and that the ice volumes on Antarctica remained relatively small until the Middle Miocene⁴. Ice-rafted debris in cores of Eocene and Oligocene age from the Southern Ocean off West Antarctica¹⁴ record limited glaciation at this time, probably associated with alpine glacial development of West Antarctica¹⁵, although others have suggested the presence of substantial thicknesses of ice since the beginning of the Oligocene¹⁶. In East Antarctica, ice conditions in the Palaeogene are unknown, but if glaciers were present, they did not reach sea level^{17,18}.

The drop in bottom-water temperatures in the earliest Oligocene caused a major crisis for the bathyal to abyssal benthonic fauna of the World's oceans including ostracods² and benthonic foraminifera¹⁹. The impact on planktonic fauna and flora was less dramatic but a simultaneous decrease (beginning in the late Eocene²⁰) in surface water temperatures^{4,11-13} caused a major reduction in diversity in planktonic organisms and a change in shallow-water echinoid fauna of South Australia²¹ although these proceeded much less rapidly than in the deep-sea regions. A low diversity planktonic realm resulting from this crisis continued to the late Oligocene after which an increase in temperature resulted in increased diversity²². The planktonic microfossils at site 277 show no dramatic changes associated with the cooling^{9,10}.

It is now well established that the frequency of breaks or gaps in the stratigraphic record in all ocean basins reaches a maximum close to the Eocene-Oligocene transition²³⁻²⁵. There are also strong indications that the erosional event that created this disconformity commenced close to the Eocene-Oligocene boundary^{7,24} and therefore is almost certainly linked with the development of Antarctic bottom water near the Eocene-Oligocene boundary. It is inferred that increased bottom-water activity caused erosion of deep-sea sediments in some areas and increased calcium carbonate dissolution in others, leading to mass wasting of calcareous sediments. The presence of widespread older disconformities^{23,25} shows, however, that deep-sea erosion is not uniquely tied to the production of cold Antarctic bottom water.

Increased oceanic turnover, inferred to have commenced in the earliest Oligocene, is reflected by increased calcareous biogenic productivity in the central Pacific causing an increase in accumulation rates^{26,27}. A major and apparently rapid deepening also occurred at this time in the calcium carbonate dissolution boundary creating a sharp contrast between carbonate-poor late Eocene and carbonate-rich early Oligocene deep-sea sediments^{26,28,29}. Major palaeoenvironmental changes even occurred in shallow marine sequences related to extremely widespread shallowing of facies³⁰, although the causes of this sea-level lowering are unknown.

It is not clear what caused the rapid cooling of Antarctic waters 38 Myr ago. The Cainozoic palaeoclimatic development of Antarctica is marked by rather rapid temperature decreases superimposed on a steady climatic cooling after the early Eocene⁴. The Eocene-Oligocene boundary event therefore probably represents a critical threshold level in Antarctic climatic development, which in itself is related to more gradual isolation of the continent, resulting from the northward spreading of Australia and the opening of the Drake Passage. Intense circum-Antarctic flow did not develop until much later in the late Oligocene when the South Tasman rise split from Antarctica^{7,31}. The timing of the opening of the Drake Passage is unknown, but is possibly linked to the palaeoglacial event 38 Myr ago^{21,28}. Without additional Antarctic deep-sea drilling, the basic causal mechanisms of such Antarctic climatic changes will remain unanswered. In the meantime the events that

occurred near the Eocene-Oligocene boundary will remain among the most profound for the entire Cainozoic.

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Grenville age for rocks in the Moine of north-western Scotland

THERE is considerable debate concerning both the age of sedimentation of the Moine Series and its tectonometamorphic history. Though several workers have argued for the presence of Precambrian metamorphic and tectonic features within the Moine (see, for example, ref. 1), others have considered these features to be essentially of Caledonian age (see ref. 2). In consequence, syntheses of the evolution of north-western Scotland during Proterozoic and early Phanerozoic time include the Moine either as an older part of the Dalradian sedimentary prism which became involved in both early and late Caledonian orogenic activity³, or as a pre-Dalradian sediment wedge affected by pre-Dalradian (Morarian) subduction⁴.

In spite of the growing number of mineral ages from several parts of the Moine outcrop which lie between 600 and 820 Myr (see, for example, ref. 1) and a "preliminary isochron indicating" a Rb-Sr whole rock age of 810 Myr (ref. 5) for the Ardour Gneiss, there is no firm, published,

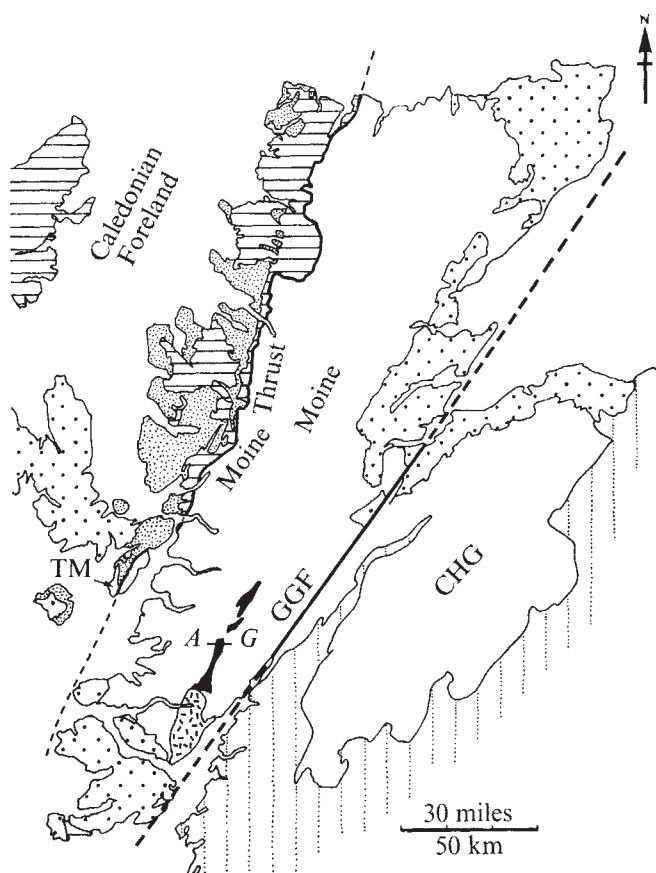


Fig. 1 Geological setting of the Ardour Gneiss. Black, Ardour Gneiss; A-G, location of sampling traverse; GGF, Great Glen Fault; coarse dots, cover and younger rocks; dotted lines, Dalradian; fine dots, Torridonian; horizontal lines, mainly Lewisian; CHG, Central Highland Granulites; TM, Tarskavaig Moine.

isotopic evidence for either the age of sedimentation of the Moine or the antiquity of subsequent orogenic activity.

Data are presented here for a Rb-Sr whole rock isochron for the Ardour Gneiss at Glenfinnan (Fig. 1), which suggest strongly that the sedimentation of the Moine and the early thermal events which affect them, are of Grenville age⁶.

The Ardour Gneiss is a large body of migmatitic gneiss which has been considered as a metasomatic derivative of Moine metasediments⁷⁻⁹. The suggestion that it is a deformed and metamorphosed pre-orogenic granite has not gained support⁸⁻¹⁰. The gneiss has a generally concordant and transitional contact with surrounding high grade, migmatitic Moine metasediments^{7,8} and it occupies the core

of a major second phase fold⁸. Neither Harry⁷ nor Dalziel and Johnson⁸ consider the gneiss to be diapiric but rather the result of partial fusion and metasomatism *in situ*. Structural elements contained by the gneiss indicate that metasomatism (migmatization) took place after the first regional deformation of the Moine but before the second^{8,10}. The migmatization is thus coeval with the acme of the early regional metamorphism which preceded and overlapped these second phase movements^{8,10}.

Eleven whole rock samples of the Ardour Gneiss were collected along a 1.5-km road section east of Glenfinnan (Fig. 1). Seven of these weighed between 16 and 30 kg, the remaining four between 2 and 3 kg. The Rb and Sr concentrations were determined using X-ray fluorescence spectrometry; ⁸⁷Sr/⁸⁶Sr ratios by mass spectrometry.

The data obtained (Table 1) define an isochron (Fig. 2) giving an age of $1,050 \pm 46$ Myr and an initial ⁸⁷Sr/⁸⁶Sr ratio of 0.708 ± 0.002 . This is the age of the last strontium isotope homogenisation, which we consider occurred during the migmatization. Following the methods of Hurley *et al.*¹¹, and Faure and Hurley¹², it can be calculated that the parent material of the Ardour Gneiss would have had a mantle ⁸⁷Sr/⁸⁶Sr ratio of 0.700, 1,250 Myr ago. On this basis Moine sedimentation must have occurred 1,250–1,050 Myr BP.

If the conclusions concerning the derivation of the Ardour Gneiss relative to the tectonometamorphic history of the Moine are correct it follows that the main, early, metamorphism took place about 1,050 Myr BP. Because it is associated with the second phase of deformation of the Moine¹³ both the first and second major tectonic events affecting these south-western, or Morar, Moine are not only of Precambrian age but are broadly equivalent to similar events in the Grenville Province of Ontario¹⁴ and the Gothides of Southern Norway¹⁵. If the age of sedimentation of the Stoer Group¹⁶ of the Torridonian can be considered as 995 ± 24 Myr (ref. 17) it is likely that Moine sedimentation was earlier.

It is significant that there is, as yet, no evidence for pre-Dalradian tectonothermal events affecting the Central Highland Granulites¹⁸, supposed equivalents of the Moine lying to the south-east of the Great Glen Fault (Fig. 1). It seems that the Central Highland Granulites are much younger than the Moine *sensu stricto*¹³ and are possibly equivalent to the Torridon Group¹⁶ of the north-western forelands of the Caledonian Belt; perhaps to the Tarskavaig Moine¹⁹ of the Kishorn Nappe; and to the Moine-like rocks of northern Mayo²⁰. On the other hand, the Moine *sensu stricto*, may be equivalent to rocks of similar aspect in Ireland, namely those of the Ox Mountains²¹ and Lough Derg²², for which a Grenville age has already been suggested²¹.

The implications in terms of crustal movements in north-western Scotland during the Proterozoic are that the Moine

Table 1 Analytical data for whole rock samples of the Ardour Gneiss

Sample no.	Sample weight (kg)	Rb (p.p.m.)†	Sr (p.p.m.)†	⁸⁷ Rb/ ⁸⁶ Sr‡	⁸⁷ Sr/ ⁸⁶ Sr‡
Mo 42 (1)*	22.6	181	123	$4.28 \pm 0.5\%$	$0.7704 \pm 0.1\%$
Mo 43 (2)	26.5	176	147	$3.48 \pm 0.4\%$	$0.7581 \pm 0.1\%$
Mo 46 (3)	25.0	167	202	$2.40 \pm 0.5\%$	$0.7441 \pm 0.1\%$
Mo 46a (4)	2.20	171	204	$2.44 \pm 0.6\%$	$0.7441 \pm 0.1\%$
Mo 46b (5)	2.76	165	219	$2.18 \pm 0.4\%$	$0.7399 \pm 0.1\%$
Mo 46c (6)	3.17	156	239	$1.90 \pm 0.3\%$	$0.7359 \pm 0.1\%$
Mo 46d (7)	2.49	166	219	$2.20 \pm 0.3\%$	$0.7400 \pm 0.1\%$
Mo 47 (8)	17.6	166	136	$3.57 \pm 1.7\%$	$0.7617 \pm 0.1\%$
Mo 48 (9)	22.2	174	147	$3.44 \pm 0.5\%$	$0.7583 \pm 0.1\%$
Mo 49 (10)	17.1	172	170	$2.94 \pm 0.7\%$	$0.7517 \pm 0.1\%$
Mo 50 (11)	16.5	164	138	$3.47 \pm 0.6\%$	$0.7610 \pm 0.1\%$

*Numbers in brackets show number on Fig. 2.

†Approximate value.

‡Analytical errors derived from replicate XRF analyses and the overall reproducibility of ⁸⁷Sr/⁸⁶Sr measurements. These were used in the calculation of the regression line²³, which has a value of 1.1 for $S/(N-2)$ (ref. 24), fulfilling the criteria for an isochron.

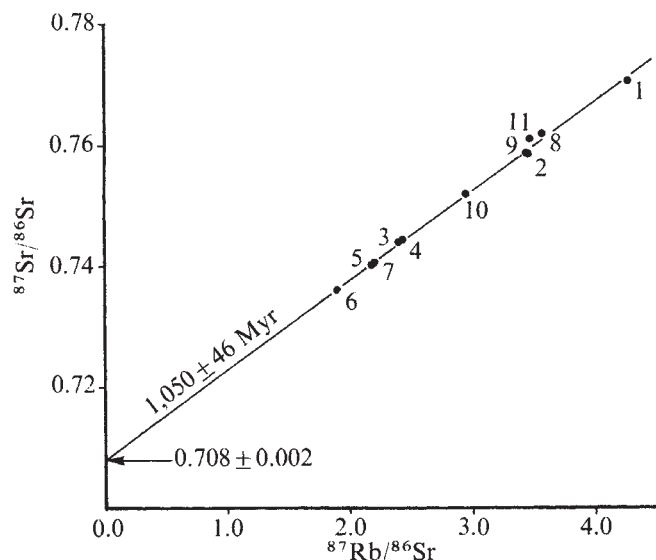


Fig. 2 Whole rock Rb-Sr isochron, errors quoted at the 2σ level. The ^{87}Rb decay constant is taken as $1.39 \times 10^{-11} \text{ yr}^{-1}$.

achieved its orogenic condition some 600 Myr before the acme of subsequent Caledonian activity. Thus consideration of the Moine and Torridonian as broadly time equivalent sedimentary prisms on the western margin of the precursor to the Atlantic Ocean³ is no longer tenable. The Stoer and Torridon Groups may have been deposited on a separate continental block from the Moine⁴, but equally the Stoer Group could be considered as molasse derived during the Morarian orogen.

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Pre-Palaeozoic basement of the Scottish Midland Valley

THE tuffs and agglomerates associated with Carboniferous volcanism within the Scottish Midland Valley comprise predominantly basic volcanic and unmetamorphosed sedimentary fragments. For the most part, the former can be confidently ascribed to the contemporary basaltic volcanism whereas the sedimentary materials seem to be derived from Carboniferous and possibly Devonian strata. In spite of the fact that the bulk of the clasts within the volcanic vents are undoubtedly of shallow crustal origin and are unlikely to have had a vertical travel of more than a few thousand metres, others—in particular the spinel lherzolite clasts known from several vents—have probably arisen from sub-crustal levels. In the Elie Ness vent, high pressure pyroxenes and pyrope garnets indicate rapid elevation from depths in excess of 60 km (ref. 1). It would be surprising if such violent, gas-fluidised eruptions, capable of carrying mantle materials, entirely failed to sample lower levels of the crust. We have to call attention to a previously undescribed assemblage of metamorphic rocks at the Partan Craig vent [NT 567852] on the southern side of the Firth of Forth, some 1.5 km east of North Berwick Harbour. The locality is situated roughly midway between the lines of the Southern Upland Fault and the extended line of the Pentland Fault.

The clasts within and around the vent comprise mainly altered alkali basalt and basaltic tuff, with a wide variety of quartzitic sandstones and scarcer cherts, limestones and indurated shales. Some relatively coarse-grained biotite-rich mafic and ultramafic clasts also occur and include (altered) mica-peridotite and glimmerite. One clast seems to be an intensely carbonated (mica-poor) peridotite.

In addition, coarse-grained gneissic fragments may be found sparingly among the pyroclasts, the largest samples up to 1,500 cm in size. From a thin-section examination of 30 samples, two main categories may be distinguished. First, garnetiferous quartzofeldspathic gneisses; second, less siliceous (but still quartz-bearing) plagioclase-clinopyroxene gneisses. The larger blocks show a foliation with flattened quartz grains parallel to a model mineralogical layering, and the whole metamorphic assemblage may well have been derived from a coarsely banded basic and acid gneiss formation.

The feldspars range from sodic labradorite to oligoclase and, like the quartzes, display strain textures. In several clasts, string perthite is present. A preliminary examination of garnet from one clast indicates a composition approximately midway along the pyrope-almandine join. Kelyphitic rims to the garnets are ubiquitous. The pyroxene is a dull greenish augite ($\sim \text{Mg}_{55}\text{Fe}_{18}\text{Ca}_{45}$) which has been extensively replaced by carbonate and chlorite. Biotite, apatite, rutile and zircon are common accessories.

These features strongly suggest granulite facies metamorphism and recall the gneisses recently described by Strogon² from Carboniferous agglomerates in central Ireland. Though the age of these gneisses is as yet unknown the metamorphic grade is clearly higher than that seen in any of the Lower Palaeozoic rocks of southern and central Scotland, and the mineral assemblages are unlike those of the Dalradian rocks of the Grampian Highlands. This strongly suggests that the gneisses were brought up from a pre-Palaeozoic 'basement'. It is worth noting that, in spite of extensive search, no clasts were found in the vent that could be unequivocally assigned to the Lower Palaeozoic greywacke-shale successions of the Southern Uplands or Pentland inliers, the Lower Devonian basalt-andesite trachyte-rhyolite succession of the Pentlands, the Dalradian metasedimentary-metavolcanic successions, or to any equivalents of the Ballantrae spilite-serpentine-blueschist com-

plex. Though too much weight must clearly not be given to such negative evidence, the possibility should be considered that at North Berwick, Upper Palaeozoic sediments directly overlie a gneissic basement complex. The biotite-rich mafic and ultramafic clasts are probably genetically related to the basaltic magmas the degassing of which was responsible for the drilling of the vent.

The discovery of gneissic blocks at North Berwick lends support to the views expressed by Kennedy³ and George⁴ that the Dalradian Basin was bounded to the south by a Precambrian landmass underlying the Midland Valley and the region further south. In north-western Ireland, upper Dalradian (mid-Cambrian) turbidites could have been derived⁵ from an uplifted basement of amphibolite and granulite facies lying to the south-east, of which the Deer Park Complex formed a part. Church and Gayer⁶ also considered that some of the Connemara metamorphic rocks could represent exposures of a sialic basement to the Midland Valley structural unit. The concept of continental crustal rocks underlying the Southern Uplands and their Irish (Longford Down) continuation has both geological and geophysical support^{7,8} and the new observations from Partan Craig would seem to confirm that a high-grade metamorphic complex also underlies at least a portion of the Midland Valley. It is difficult to reconcile the presence of these gneissic clasts with the various models proposed by Jeans⁹, Gunn¹⁰ and Mitchell and McKerrow¹¹, in which post-Arenig formation of the Midland Valley are postulated to overlie an earlier Palaeozoic oceanic crust.

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Cromagnon in the Iron Gate Gorge of the Danube

RADIOCARBON datings on human skeletons from the Padina site in the Iron Gate Gorge of the Danube in Yugoslavia have revealed that they belonged to a population at least 9,000 yr old. This age is of especial importance because it is the earliest yet obtained by radiocarbon analysis for human bones from the region. It indicates for the first time the existence of a Cromagnon population in Yugoslavia.

During excavations at Padina¹, the remains of 37 individual skeletons, all in a very bad state of preservation, were uncovered. Anthropological study and laboratory examinations have shown that the skeletons possess all the morphological characters of the Cromagnon racial type. The skulls are long with huge supraorbital ridges and low vaults and the jaws are very large, with very rough areas for muscular attachments and with large prominent ridges and spines. The bones of the limbs are also large and robust with strongly marked muscular imprints. The average esti-

mated height of the male individuals is approximately 180 cm (ref. 2).

Bone tissue for the analysis reported here came from the upper end of the best-preserved femora obtained. The specimens came from the lowest geological layer of the excavations. So far, two dates have been obtained: $6,487 \pm 83$ yr b.c. (BM1144); and $7,248 \pm 103$ yr b.c. (BM1147).

The Padina population lived in arc-like hollows beside the river bank which was intersected by rocky ridges and surrounded by wooded cliffs. Alterations in the course of the river destroyed several such settlements, and gravely impaired the state of preservation of the human remains.

The excavations are into early Holocene deposits (~10000–6000 bc). At that time the climate, flora and fauna, and the general terrain and geography of Yugoslavia, became similar to those of the present day. The permanent snow cover of the Dinaric mountains melted, and grassland—bringing with it wild wheat, oats, barley and rye—spread along the Danubian plain. Wild goats, pigs, cattle and deer inhabited the forest³. It is very probable that in this region some Pleistocene hunters and gatherers adapted themselves to the new climatic conditions and migrated along the Danube, which offered them a new source of food. Fishing became as useful as gathering and hunting.

Archaeological finds and cultural remains at Padina (and other sites such as Hajdučka, Vodenica, Lepenski Vir and so on¹ have not previously provided suitable material for accurate dating, although a radiocarbon date of 4,950 yr b.c. has been obtained from charcoal from the floor of one of the houses at Lepenski Vir (British Museum Radiocarbon Laboratory). Recently, similar material from Vlasac (not very far from Padina), examined at the Radiocarbon Laboratory in Zagreb has given a date of $5,609 \pm 93$ yr b.c. (ZG 267); until this report, that was the earliest estimated age for these populations.

It is expected that further excavations further down the River Danube in the near future will reveal more human material in a better state of preservation, thus enabling a more detailed study of this newly discovered Cromagnon population.

I am grateful to the British Museum Research Laboratory for performing the ¹⁴C analysis, and to the Radiocarbon Committee for financial support. I also thank the Institute of Archaeology in Belgrade, Yugoslavia, for providing the financial support for the field work and anthropological study.

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Effect of sewage sludge on mineralisation of organic carbon in soil

SOME 40% of the sludge produced at inland sewage works in England and Wales is applied to agricultural land¹. In addition to the plant nutrients and organic matter they provide, such sludges also contain various quantities of heavy metals². In some cases, repeated and very heavy dressings of sludge have led to accumulations of harmful elements that are toxic to plants³. There has been concern about their possible accumulation in agricultural soils receiving sludge⁴.

Table 1 Sludge analysis

	Consolidated activated sludge (AS)	Secondary digested sludge (DS)	Vacuum filter cake (FC)
Solids*	2.6	11.1	23.6
Organic matter†	67.8§	62	62
Macronutrients ‡N	4.80	2.63	3.39
P	2.54	1.97	2.27
K	1.41	0.25	0.24
Trace elements ‡Zn	2,652	4,225§	5,650
Cu	758	730	990
Ni	30§	60§	340

*% wet weight.

†% dry matter.

‡p.p.m. dry matter.

§Figure for similar but not identical sample.

since, as Tyler⁵ suggested, microbial activity in soils is susceptible to enhanced levels of heavy metals. The state of soils receiving sludge is monitored by regular analyses⁶, and the Advisory Services of the Ministry of Agriculture (ADAS) have recommended a combined upper limit⁷ to the total load (pre-existing and applied) of Zn, Cu and Ni, above which crop plants may be affected. We have investigated whether the approved quantities of sludge have adverse effects on the microorganisms responsible, in aerobic conditions, for mineralising the organic carbon in the soil, and have shown that three contrasting sludges from the same works, when mixed with soil in amounts up to five times the recommended limit, had no adverse effect on carbon mineralisation.

The three sludges tested (Table 1) came from a treatment plant receiving mixed domestic and industrial effluent, and they have all been used agriculturally. A single potting soil was chosen for this investigation because its properties (loam, pH 6.7, organic matter 4.1%) placed it near the centre of the range of agricultural soils. It was air dried and sieved through a 2-mm mesh. Samples of soil were treated with quantities of sludge—roughly equivalent to multiples of the maximum amount likely to be applied to agricultural land in one year (Table 2, level I)—thoroughly mixed, and placed in 15-cm pots. Maximum applications of two of the sludges (activated and secondary digested sludge) were limited by their high liquid content. Table 2 describes the sludge applications in three ways: as the actual amount of sludge added per treatment (A); as the ratio of the combined load of Zn, Cu and Ni (calculated according to ADAS procedure) to the recommended maximum load (B); and as the amount of organic matter added, expressed as a percentage of total soil (C). It should be noted that the three highest rates initially increased the soil organic matter by 25–250%.

The pots were planted with barley and set up in an illuminated greenhouse (minimum temperature 10 °C) for

nearly a year, during which three crops were grown to the five-leaf stage and harvested. The barley was watered regularly, and the drainings recycled. The soils were allowed to drain and to dry out between crops and after the last crop. At this time samples of soil were taken from each pot, air dried, and again sieved through a 2-mm mesh. Their pH in water then ranged from 6.0 to 6.4.

Duplicate aliquots (10 g) of each sample were mixed with 0.5 g of ground oat straw (< 1 mm, 0.68% total nitrogen and 40.1% organic carbon), brought to 50% saturation with water containing ammonium nitrate to provide 100 p.p.m. N in the dry soil, and incubated at 30 °C. Carbon mineralised as CO₂ was determined by the barium peroxide method⁸ and expressed as p.p.m. of air-dry soil.

After 76 d incubation the pH in water ranged from 5.7 to 6.3, and soluble nitrogen (NO₃⁻ and NH₄⁺ extracted with 1 M KCl) from 93 to 151 p.p.m. dry soil, neither being related to sludge treatments. Clearly, carbon mineralisation had not been retarded by low pH or lack of available nitrogen. Figure 1 presents the cumulative CO₂-C release after 14, 35, 50 and 76 d. The lack of a consistent pattern in the results from the 0–III treatments indicates the degree of intra-treatment error.

There are only small differences between sludges and heavier applications of sludge increase the quantities of CO₂ produced, presumably in part from the organic carbon they contain. Since a parallel comparison showed no significant difference between the CO₂ produced by equal applications (350 g per pot) of filter cake, one of which had been first ignited to destroy its organic matter, it seems unlikely that the effects of the higher applications can be explained as reduced microbial activity on increased organic matter. So it seems that the overall activity (as measured by CO₂ production) of the organisms responsible for the decomposition of natural and added organic matter is unaffected by any of the types and levels of sludge applied.

Our highest sludge additions raised the copper concen-

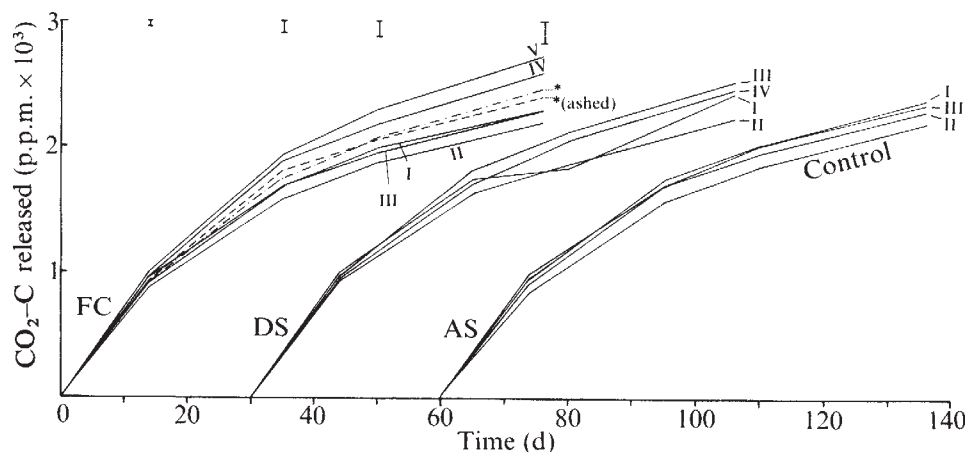


Fig. 1 Carbon mineralisation, measured as p.p.m. of C released as CO₂, in soil treated with increasing amounts (I–V, see Table 2) of consolidated activated sludge (AS), secondary digested sludge (DS) and filter cake (FC). Three control soils have been averaged. The pot treatments were not replicated and the least significant differences ($P < 0.05$) shown relate only to the two samples from each pot that were incubated separately. *Indicates a subsidiary comparison of ashed and unashed 350-g samples of FC.

Table 2 Sludge treatments

Level	A			B			C		
	AS	DS	FC	AS	DS	FC	AS	DS	FC
0	0	0	0	0	0	0	0	0	0
I	310*	75*	35†	0.08	0.11	0.18	0.29	0.3	0.3
II	630	150	70	0.16	0.22	0.36	0.6	0.56	0.55
III	1,060	300	140	0.27	0.44	0.72	1.0	1.12	1.11
IV	—	590	280	—	0.88	1.44	—	2.19	2.21
V	—	—	1,000	—	—	5.13	—	—	9.91

A, As the amount of sludge added per pot; B, as multiples of the ADAS maximum load of Zn, Cu and Ni; C, added organic matter as % of dry soil.

*ml.

†g fresh weight.

tration in the soil by 123 p.p.m., whereas Tyler⁵ reported that the mineralisation of soil N may be adversely affected by copper concentrations greater than ~ 50 p.p.m. of dry soil. Nitrogen mineralisers are, however, thought to be more susceptible than carbon mineralisers, and Tyler's soils were very acidic (much more so than most agricultural soils in Britain). Heavy metals are widely reported to be more harmful in acidic conditions, so we do not find his or our results inconsistent.

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Human olfactory communication

It has long been known that animals use their olfactory senses to communicate information, including sexual status, individual identification and maternal attraction^{1–3}. Olfactory communication has been demonstrated throughout the Mammalia, including the primates^{4,5}, and there has been speculation as to whether or not it exists in some form in man^{6,7}. Substances are known to exist on man that serve an olfactory function in other animals⁸ and there are many apocrine and sebaceous glands on the human body that produce such secretions. We have done two experiments, the first to determine whether adults can identify an individual and determine his or her sex by the odour of an article of clothing, and the second to examine whether an infant can identify its mother's odour by a behavioural response.

In the first experiment the subjects were 29 freshmen college students, 16 male and 13 female, all recruited by announcing the nature of the experiment in class and asking for volunteers. The subjects were asked not to use any soap, perfume or deodorant for 24 h before the experiment and to wash only with clear water during this period. Then

they were each given a plain white, appropriately sized, T shirt of 50% polyester and 50% cotton, and asked to wear the shirt as an undergarment for 24 h. The subjects were asked to don the shirts after class and remove them before class the next day. They were provided with new identical sealable plastic bags in which to place their shirts. The shirts were then collected, removed from the bags, and put in wax-coated cardboard ice buckets in which a one-inch triangular hole had been cut (to allow the subjects to sniff the contents). The ice buckets were then placed on waxed paper with the shirts arranged so that the under-arm portion was closest to the hole. Each subject was tested individually in a testing room. The placement of containers was randomised for each subject.

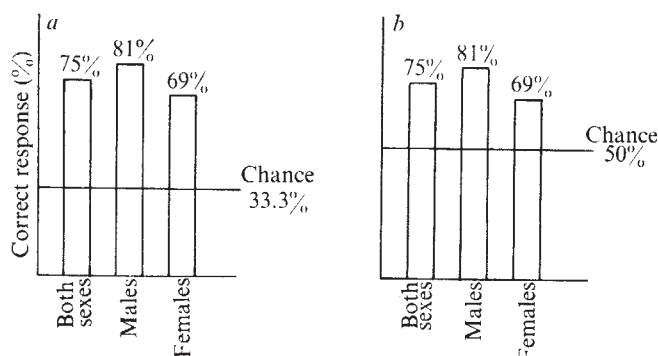
The first test was a three-choice discrimination consisting of: (1) the subject's own T shirt; (2) a strange male's T shirt, and (3) a strange female's T shirt. After each subject entered the testing room and was seated, he was asked to "sniff each bucket three times and indicate which one is yours—take as much time as you wish, and sniff as many additional times as you wish". Generally, each subject sniffed each bucket once in succession and then repeated this process.

The second test always followed the first and was the same except that it was a two-choice discrimination between the same strange male and the same strange female T shirt as used in the first test, and the subject was asked to discriminate the sex of the wearer by indicating which was male. Test 2 followed test 1, within a few minutes.

In each test, thirteen of the sixteen males and nine of the thirteen females answered correctly. This provided a highly significant result of $P < 0.001$ for test 1 and $P < 0.005$ for test 2 when a binomial expansion was used as a statistical measure.

While the number of correct responses was the same in each test, the individuals who answered correctly were not always the same. Several of the students remarked that they felt they would have been able to make the discrimination

Fig. 1 Experiment 1. a, Test 1 shows the percentage of correct responses in identifying subjects' own odour in a three-choice discrimination. b, Test 2 shows the percentage of correct responses in identifying male odour in a two-choice condition.



at a lower concentration of odour, but none of them mentioned finding either the strength or types of odour objectionable.

There was considerable variation in the concentration of the odour samples and no attempt was made to control diet, sexual cycles, or drug variations within the subjects or donors, yet most subjects could select the correct odour easily. During informal questioning after the test the male odours were usually characterised as musky and the female odours as sweet.

These tests show that at least the rudimentary communications of sexual discrimination and individual identification can be made on the basis of olfactory cues.

In the second experiment we investigated whether a similar type of ability could be demonstrated in an infant and, further, if olfactory discrimination would elicit a behavioural response. Several authors, including Darwin⁹, have provided anecdotal reports that children may use odour to identify their mothers at an early age. Pratt *et al.*¹⁰ reported that Pryer, Canestrini and Peterson Rainey in separate experiments obtained preliminary but conflicting evidence that infants may be attracted to the odour of their mothers' milk when nursing.

The subjects were recruited by asking mothers in the delivery ward of the University of California Moffitt Hospital to volunteer for the experiment. The subjects were 14 healthy full-term breast-feeding mothers (both multiparas and primiparas) and their infants. Tests were conducted on the second day, during the second week, and during the sixth week after birth. The olfactory stimulus used for these tests was obtained by asking each mother to place a breast pad (white cotton sponges) inside her bra for 3 h before testing. The mothers were also asked not to feed their babies during this period so that the infants would be somewhat hungry, but not distressed, during testing.

The test consisted of the experimenter holding a folded breast pad with thumb and index finger under the infant's nose at a distance of 1–2 cm for approximately 30 s while being careful not to touch the infant. During each test the infants were exposed to: (1) a clean moist pad; (2) a familiar (own) mother's pad, and (3) a strange mother's pad. For each infant the sequence of presentation was random. During the 6-week test the infants were also tested with a pad that had been moistened with raw cow's milk. Each test was conducted in either a standard hospital bassinet, or the infant's crib at home.

The test on day two was conducted in the hospital at the mother's bedside, and the 2- and 6-week tests were conducted in the home of the subject with the mother present. All breast pads were collected just before testing, except when it was necessary to travel between homes and then the pad from the strange mother was stored in a plastic bag. The order of presentation of pads was random.

Infants were tested while sleeping whenever possible and not tested when distressed or crying. Pilot work had indicated that this was the only time that the infants could be tested independently of visual and auditory cues. When an infant was aroused by the stimulus, time was allowed between presentations for the infant to return to its original behavioural state.

As Fig. 2 shows, during the rest on day two, only one of the 10 responded to any of the stimuli presented. That infant made a sucking response to both the strange and familiar odour.

At 2 weeks, eight of the 10 infants responded to the odour of the strange mother, seven responded to their own mother, and three infants showed a differential response between the two. A differential response occurred when the infant responded only to his/her own mother's pad and not to the strange mother's pad. In the case of the 2-week test nine infants responded, six similarly to both odours, two

just to the strange odour, one just to the strange odour and one just to the familiar odour. At no time did any infant respond to the clean moist pad.

At 6 weeks only one of the infants responded to the strange mother, seven responded to the mother's pad and six showed a differential response. The most common response observed (in five of the infants) was a sucking orienting response to the mother's pad and no response at all to the strange mother's pad. A sixth infant who was hungry and sleeping when the test began did not respond at all to the presentation of the strange mother's pad, but awoke and began to cry when its own mother's pad was presented.

In each of the 6-week tests the responses to the raw cow's milk were the same as to the strange mother's pads.

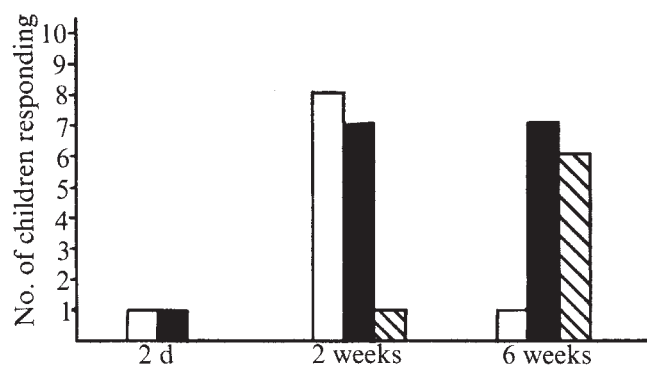


Fig. 2 Experiment 2. The responses of the 10 infants at 2 d, 2 weeks and 6 weeks to the strange mother's odour (open bars) and their own mother's odour (black bars). The hatched bar represents the number of children responding to their own mother and not to the strange mother.

Experiment two tested the infants' abilities to identify their mothers by odours left on breast pads at 2 d, 2 weeks, and 6 weeks after birth. At 2 d there was no response. At 2 weeks the infants showed general arousal and minimal, if any, discrimination. Then at 6 weeks six of the 10 infants tested could identify their own mother's odour from that of a strange mother's odour. At 6 weeks of age infants responded to their own mother's odours with a pattern of orienting and sucking that was markedly different from their response to both the strange mother's odour and the odour of raw cow's milk, which was added as an additional control. Furthermore, at least six of the infants showed a positive attraction to the mother and only one infant responded at all to the strange odour. This was a negative response, with a head jerk and a cry. The existence of olfactory maternal attraction suggests that humans have a pheromonal system and that it operates at a very early age. Since doing this work I have become aware of a strikingly similar experiment by Macfarlane¹¹ in which he was able to show a differential response at 6 d of age.

The initial identification of the mother may not be due to a response to her odours but rather to odours placed on her by the infant during earlier contacts, as demonstrated in other primates¹². Furthermore, the possibility of this maternal scent marking in humans is supported by the common observation by parents of children who reject a favourite teddy bear or blanket after it has been washed because of the loss of odour acquired from earlier contacts.

Although the source of these odours has not been clearly demonstrated, olfactory cues seem to generate behavioural responses in infants, and sexual and individual identification seems to occur in adults.

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Importance of antennae for orientation of insects in a non-uniform microwave electromagnetic field

MICROWAVES can affect living organisms in different ways¹. Radiation of high intensity is lethal, due to the thermal effect on the living tissues. Different organs are selectively sensitive both in vertebrates² and in insects³. Behavioural studies with rats have demonstrated that, in a choice situation, the animals will respond to an electromagnetic field of a rather low intensity, by leaving it⁴. Our investigations revealed that insects respond to the intensity gradient of a non-uniform microwave electromagnetic field in a similar way. In an arena illuminated by a non-uniform field insects carry out oriented movements avoiding the regions which are more intensely illuminated. In this paper we analyse the involvement of antennae in the orientation mechanisms underlying the avoidance response.

Adults of *Caryedon gonagra* (Fab.) and last instar nymphs of *Pyrrhocoris apterus* L. were used. The former species represents a beetle with low tendency to crawl, the latter one an active and fast moving bug. Antennectomised and intact

specimens were tested in a round arena constructed from a polystyrene Petri dish. The arena was placed on the broad wall (bottom) of the waveguide of rectangular cross section (Fig. 1a). In this position a symmetrically non-uniform electromagnetic field was obtained inside the arena, with maximum flux density (power density) occurring in the axial E plane of the waveguide. The insects were released in the centre of the arena and all individuals avoiding the most intensively illuminated central band of the arena were counted every 60 s for 10 consecutive min. The width of the band was arbitrarily chosen so as to enclose the region of power densities greater than 50% maximum power density (Fig. 1b). The observations were made at five different power densities and the controls observed at zero electromagnetic field intensity. Behaviour of the beetles and bugs was observed three and five times with 11 and 5 fresh specimens, respectively. The maximum numbers of individuals observed at each repetition outside the central band after cessation of response, were summed up; the figure obtained served to express the avoidance response at a given power density. The significance of differences was estimated on the basis of a χ^2 test. Mortality was expressed as percentage of dead animals at the end of the 10-min observation period. A TESLA 50SA51 magnetron was used to generate the microwave power. Incident power levels used ranged from 100 to 1,200 W in a continuous wave (CW) regime and at a frequency of 2,375 MHz. The maximum power density inside the dish for the transverse electric (TE₀₁) mode used was 3.3-40 W cm⁻².

At zero incident power the beetles *C. gonagra* (Fig. 1c) tend to remain near the site of release. At 3.3 W cm⁻² both antennectomised and intact specimens retained a tendency to remain in the central band. When electromagnetic power of increasing intensity was applied the intact animals began to respond by moving out of the central band at power densities of 6.6 W cm⁻², the avoidance response being highly significant at power densities greater than 6.6 W cm⁻². The corresponding threshold value for significance of the response of antennectomised beetles was at power densities greater than 13.2 W cm⁻². Lethal effects of microwaves were observed only at highest intensities used and predominantly in the antennectomised individuals.

Both antennectomised and intact nymphs of *P. apterus* (Fig. 1d) spread more or less equally to the periphery of the arena at zero incident power and quickly changed their positions

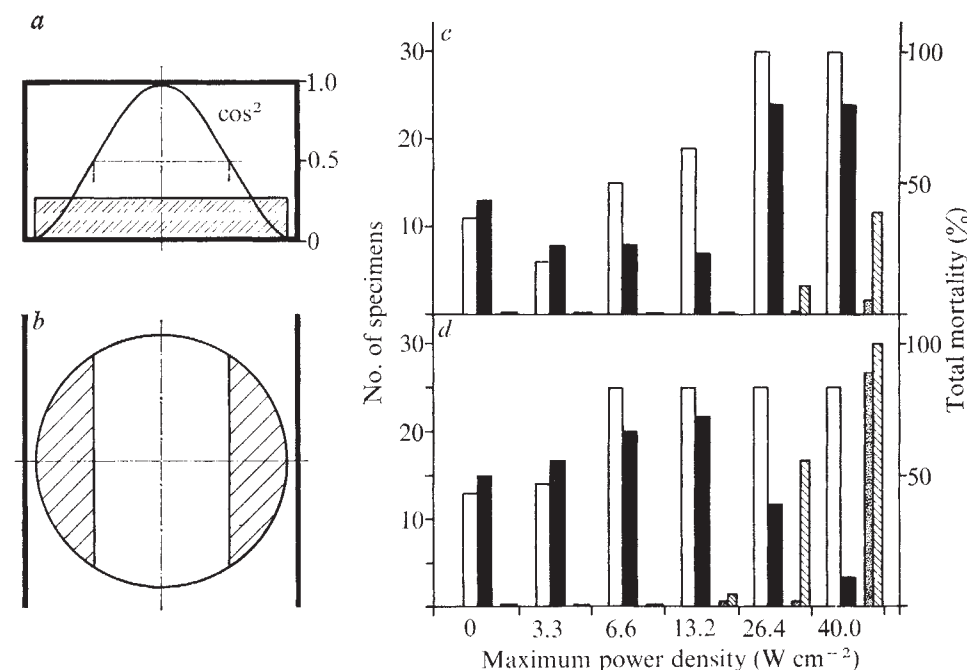


Fig. 1 a, Cross section of waveguide showing microwave energy distribution ($\cos^2$) across arena. b, Surface view of arena; central (clear) band receives power densities greater than 50% maximum. Avoidance of central band by *C. gonagra* (c) and *P. apterus* (d) in intact (open columns) and antennectomised specimens (solid columns); and mortality in intact (stippled columns) and antennectomised specimens (hatched columns).

thereafter. Significant avoidance response was recorded only in intact specimens at power densities 6.6 W cm^{-2} or more. The response in antennectomised bugs was not statistically significant, although they showed a slight tendency to avoid the central band at higher power densities. Mortality was higher in the antennectomised specimens than in the intact ones.

These experiments thus provide two lines of evidence for importance of antennae in the orientation of insects along the intensity gradient of a microwave electromagnetic field. First, the threshold incident power levels for initiating the avoidance response are higher when the antennae are lacking. Second, the lack of antennae increases vulnerability of insects to microwave energy non-uniformly distributed over the arena, apparently because of impairment of their orientation mechanisms. A certain ability, although significantly reduced, of antennectomised individuals to avoid the field of intensive microwave radiation, however, suggests that still other mechanisms of orientation, which do not depend on sensory input from the antennae, may exist. We hesitate to speculate about their nature as well as about a possible biological significance of antennal sensitivity to microwaves. The threshold densities causing aversion in intact insects are 1,000 times higher than those in rats⁴; however, they are still well below the lethal dose (J.O., J.Z. and J.D., unpublished). We suspect that in our experiments the aversion induced by microwaves is mainly due to thermal effects. Nevertheless, the sensitivity of intact specimens to rather low densities (6.6 W cm^{-2}) of the microwave field may indicate that the sensory apparatus of insects can detect, and that the animals can respond to, quantities of radiation producing no harmful effects. Non-thermal effects of microwave radiation on developing insect tissues have already been demonstrated⁵.

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Non-ageing developmental variant of *Caenorhabditis elegans*

THE study of mechanisms affecting the rate of ageing can be facilitated by naturally occurring phenomena, innate to some organisms, that enable the species to retard its ageing rate and extend its life span¹. Such a phenomenon exists in certain species of nematode. Larval forms of the free-living soil nematode, *Caenorhabditis elegans*, possess the ability to enter a semi-dormant, quiescent state referred to as the dauer larval stage (German, "enduring" larva). Newly hatched larvae of *C. elegans* undergo four larval stages (L1-L4) punctuated by

moulting. If larvae are starved, they will enter the dauer state during the second larval moult. At this time, the old cuticle is replaced by a special, relatively impermeable cuticle unique to dauer larvae².

During this state the larvae cease to feed, the pharyngeal muscles become inactive and all development ceases. Dauer larvae are thermotactically opposite to non-dauer larvae³ and are resistant to harsh chemicals such as 1% sodium dodecyl sulphate (SDS)². Although no food is ingested, the dauer larvae are capable of moving during the dauer state. Dauer larvae maintain a high degree of motility close to or greater than that of non-dauer larvae for the first 2-3 weeks after dauer formation. This motility never completely ceases but declines gradually to a minimum by the end of the dauer life span (approximately 70 d). All dauer larvae used in these experiments were moving. Placing dauer larvae in a bacterial suspension of $\geq 5 \times 10^8$ bacteria per ml will stimulate the larvae to come out of the dauer state, undergo the third and fourth larval moults, and become mature, productive adults.

Fig. 1 *Caenorhabditis elegans* var. Bristol was cultured in liquid S-medium supplemented with *E. coli* strain OP50 at 10^8 bacteria per ml as described previously⁴. All cultures were grown at $20 \pm 1^\circ \text{C}$. Initial inoculum was 6 worms per ml. 0, Time of hatching. Δ , Number of bacteria per ml as determined using Petroff Hauser counter; $\circ$, worms per ml; $\bullet$, dauer per ml as determined by resistance to 1% SDS².

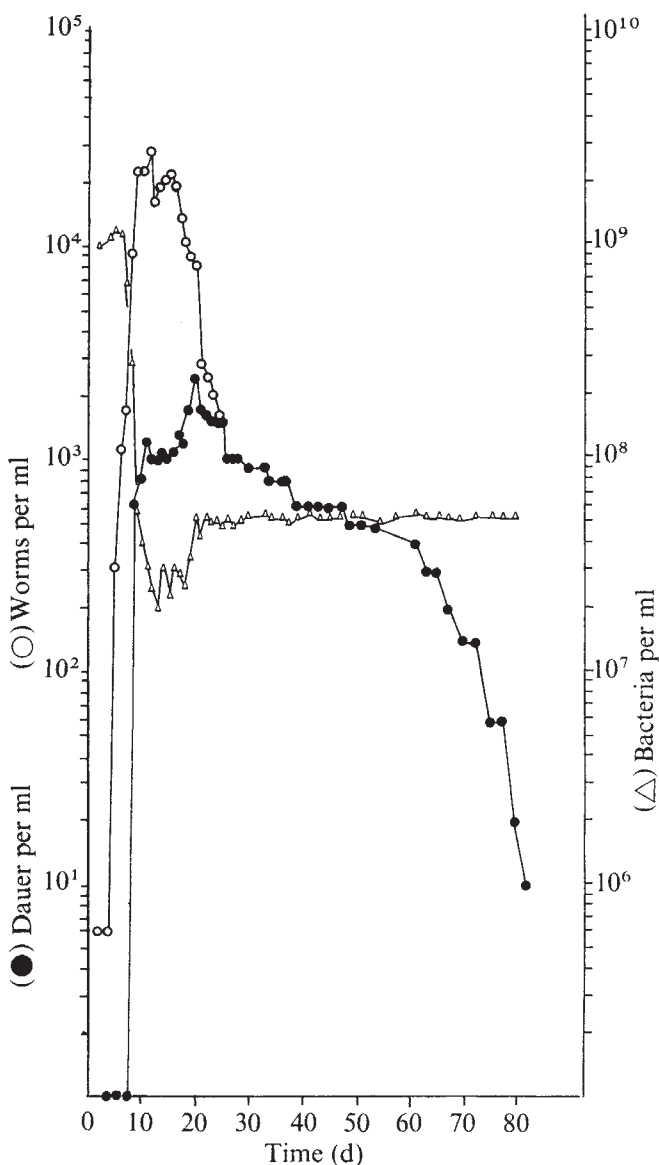


Table 1 Effect of duration of dauer state on life span

Duration of dauer state (d)	Post-dauer life span (Mean $\pm$ 95% c.i.m.*)	Mean no. of eggs	Egg viability (% hatch)	Total life span
5	11.16 $\pm$ 1.20	283	100.0	16.16
10	10.08 $\pm$ 0.96	330	98.1	20.08
15	9.68 $\pm$ 1.16	236	97.7	24.68
20	10.89 $\pm$ 1.13	304	100.0	30.89
25	10.19 $\pm$ 1.08	323	91.3	35.19
30	10.53 $\pm$ 0.93	242	93.4	40.53
35	9.38 $\pm$ 1.12	282	97.4	44.38
40	10.23 $\pm$ 1.42	270	95.0	50.23
45	9.90 $\pm$ 1.47	290	84.8	54.90
50	10.16 $\pm$ 1.71	339	100.0	60.16
55	11.05 $\pm$ 1.85	274	94.2	66.05
60	10.24 $\pm$ 1.81	394	97.7	70.24

*c.i.m., Confidence interval of the mean.

Dauer larvae were isolated by the SDS resistance method of Cassada and Russell² and were maintained in M-9 salt solution⁴. At 5-d intervals samples of 100 dauer larvae were removed and placed on NGM agar plates seeded with a drop of *E. coli* (10^9 ml⁻¹)⁴. Percentage survival, number of eggs laid and egg viability were determined at daily intervals at 20 °C. The mean life span of nematodes that never entered the dauer state at 20 $\pm$ 1 °C is 12.76 $\pm$ 1.08 d (n = 50) and the average number of fertilised eggs produced per parent is 280 $\pm$ 40 ($\pm$ 95% c.i.m.*; n = 25). Normal egg viability is $\geq$ 97%.

Dauer larvae can be maintained for more than 70 d (Fig. 1). The 50% survival time is approximately 45 d at 20 °C. There is a small increase in the frequency of post-dauer mortality (Fig. 2). As the duration of the dauer state increases, the percentage of larvae which fail to recover from the dauer state increases from 0% at 10 d to 32% at 60 d. This effect is probably due to the reduced vitality brought on by starvation.

The duration of the dauer state has no effect on the post-dauer life span (Table 1). There is no observable effect of the dauer state on (1) post-dauer life span; (2) post-dauer egg production; or (3) egg viability. Worms that have been in the dauer state for 60 d have the same post-dauer life span, lay the same number of eggs, and have the same egg viability as worms that have been in the dauer state only 5 d.

Dauer larvae have a primordial gonad composed of approximately 35 nuclei. The duration of the dauer state has no effect on the life span of the F₁ progeny which are descendants from these germ cells (Table 2).

If ageing in *C. elegans* is a result of random accumulation of damage (nuclear or cytoplasmic) due primarily to external insult for example, ultraviolet, X rays, cosmic rays, and so on, then it is reasonable to presume that prolonging the dauer state would be correlated to an increase in accumulated damage and subsequently a decrease in the post-dauer life span. This result was not found. There was no detrimental effect on life span observed by extending the dauer state up to 60 d. Mean post-

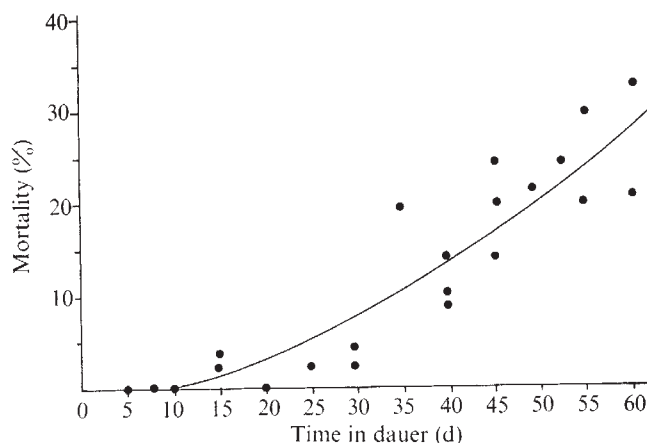


Fig. 2 At 5-d intervals, samples of 50 dauer larvae (isolated by SDS resistance²) were removed and placed on NGM agar plates seeded with a drop of *E. coli* (10^9 ml⁻¹)⁴. Percentage mortality was calculated from the proportion of dauer larvae that failed to recover from the dauer state and died within 2 d of feeding.

dauer life span remained constant and the total life span simply increased by the amount of time spent in the dauer state.

One might expect an increased probability of accumulated damage in the germinal nuclei and cytoplasm and subsequently a reduction in the viability and mean life span of F₁ progeny because the primordial gonad is present throughout the dauer state. This effect was not observed. Egg production, viability and F₁ life span remained constant. It must be concluded that because there is no life-shortening effect of prolonging the dauer state up to 60 d (four times the normal life span) externally induced damage is not a primary cause of ageing in *C. elegans*. Normal ageing therefore must be an innate process whose rate is directly related to the metabolic rate.

The phenomenon of dauer larvae has been observed and described for many years. Many parasitic forms have infective larvae which can survive for extended periods in a dauer-like state. If they contact a host and subsequently develop, there is no correlation, above a survival threshold, between "age" of the larva and its ability to become an adult⁵. Our observations with *C. elegans* parallel the results obtained with parasitic forms.

Dauer larvae seem to be able to reduce or suspend the rate of ageing. This "non-ageing" quiescent state offers an interesting model for the study of mechanisms that control the ageing process. Analysis of the developmental processes that enable a metabolically active, rapidly growing larva of *C. elegans* to

Table 2 Life span of progeny of experimental worms

Duration of dauer state (d)	F ₁ Life span (Mean $\pm$ 95% c.i.m.*)	Mean no. of eggs
5	11.10 $\pm$ 1.29	275
10	12.47 $\pm$ 1.02	279
15	11.70 $\pm$ 1.19	198
20	11.59 $\pm$ 1.01	224
25	10.13 $\pm$ 0.94	269
30	11.44 $\pm$ 0.75	294
35	11.02 $\pm$ 1.15	198
40	11.70 $\pm$ 1.33	169
45	11.52 $\pm$ 1.18	264
50	11.78 $\pm$ 1.37	273
55	11.53 $\pm$ 1.21	199
60	10.77 $\pm$ 1.32	248

*c.i.m., Confidence interval of the mean.

Samples of 100 F₁ larvae were taken on the second day of egg laying from parents who had been in the dauer state for increasing durations. The life span of the F₁ larvae and the average number of fertilised eggs produced by the F₁ worms were measured.

enter a developmentally dormant, "non-ageing" state may offer valuable insight into the controlling mechanisms of ageing.

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Graphs of contracting glycerinated *Amoeba proteus*

GLYCERINATED *Chaos-Proteus* amoebae preserve their normal shape, but will contract after washing and introduction of physiological concentrations of ATP, Ca^{2+} , and Mg^{2+} (ref. 1). We have recorded the motion of activated glycerinated amoebae (amoeboid models) in a manner such that endoplasmic motions, shape changes, and contractile sites are pictorially graphed by the model during its contraction. Our findings contradict the frontal-zone contraction hypothesis, but do not unequivocally support the ectoplasmic tube contraction theory.

Glycerination of *Amoeba proteus* was carried out in 50% glycerol (v/v), EDTA 5 mM, Tris-HCl buffer 0.01 M, KCl 0.05 M, pH 7.0, at $\sim 10^\circ\text{C}$. It is critical to reduce the medium to a small volume containing a few starved amoebae and then rapidly to apply a large volume of this solution. Glycerination was continued for at least 24 h and its quality was judged by retention of amoeboid shape and by subsequent contractility of the model. Actual contractions of amoeboid models were in 25% glycerol (v/v) with ionic contents and buffers as above plus ATP, Ca^{2+} , and Mg^{2+} , each at 0.5 mM. The time for contraction varied from 1 to 10 min following ATP-Ca-Mg application.

Contractile dynamics were recorded under darkfield microscopy at low power ($\times 60$), and photographs were taken sequentially at 1-min exposures with ~ 5 -s intervals. Figure 1 is a sequence of four photographs recording movements of amoeboid model contraction. The first photograph shows the amoeboid model washed immediately after adding

ATP-Ca-Mg. Cytoplasmic granules appear white in the photographs and their horizontal displacement is recorded as streaks whose lengths vary with their velocities². Granules that are moving vertically will show only a sharp single image. The beginning of contraction (second photograph) is indicated by the streaks of granules in motion. Their velocity can reach $50\ \mu\text{m}\ \text{min}^{-1}$. The third photograph in this sequence illustrates the continuing contractility of this model. The last photograph is the contracted amoeboid model, indicated by a lack of recorded motion.

Study of these photographs facilitates precise determination of the contractility sites and the resultant motion of this model. The outlines below the fourth photograph summarise the visible changes in shape displayed by this glycerinated model. Measurement of the large leading pseudopodium reveals a slight change in length, emphasising that contraction occurs predominantly in the width of the model. Ectoplasmic contraction towards the central portion would account for this observed motion.

Figure 2 is a three-photograph sequence of a glycerinated model illustrating that pressure can be generated by contraction, as indicated by endoplasmic displacement. In the first and second photographs, at pseudopodial junctions near the tail end (bottom of photograph), prominent streaks can be seen in the central portion of the model, indicating endoplasmic motion. The volume change during contraction of this model is relatively small. The last photograph of this sequence records the endoplasm being forced out of an ectoplasmic rupture into the environment.

These photographs of ATP-Ca-Mg-activated *A. proteus* models demonstrate that contraction first, is localised in the ectoplasmic tube; second, can displace glycerinated fluid-like endoplasm at a velocity reaching $50\ \mu\text{m}\ \text{min}^{-1}$; and third, can generate a pressure within these models displacing the fluid-like endoplasm.

The ectoplasmic tube contraction theory (ETC)³ requires that ectoplasmic tube contraction produces a pressure which displaces relatively non-contractile endoplasm; these specific requirements are supported by this investigation. Further, during normal amoeboid motion, the ETC theory predicts some interconversion of ectoplasm to endoplasm at its interface as a part of the contraction process. Ectoplasm to endoplasm interconversion may occur once, but it is not a continuous feature during contraction of glycerinated models. Reversible relaxations and contractions of these models were not achieved. Amoeboid models can contract, but they do not exhibit organised amoeboid motion, which might be explained by the lack of ectoplasm to endoplasm interconversion.

Frontal endoplasmic contractions were never observed in our studies, which conflicts with the fundamental contractile requirements of the frontal-zone contraction hypothesis^{4,5} for amoeboid motion.

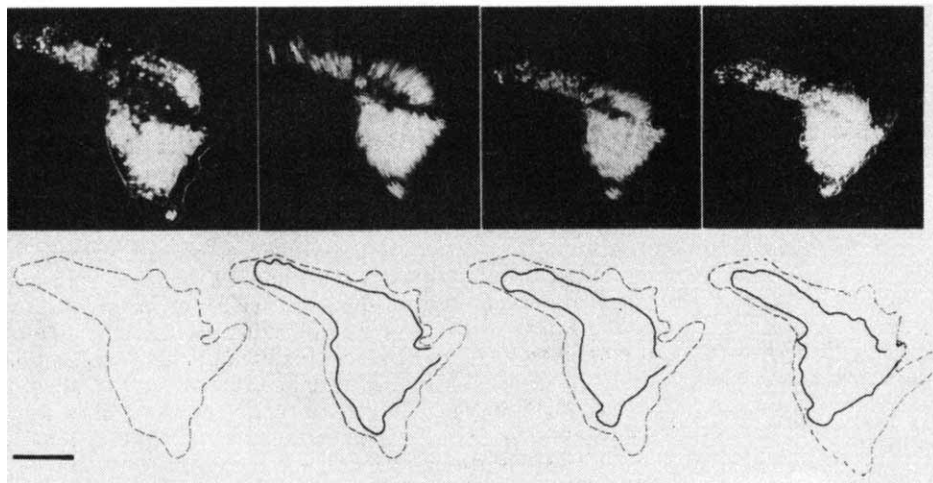


Fig. 1 Graphic sequence of a single glycerinated model contracting: ----, Before contraction; —, outline of contraction state of photograph directly above it. The bar represents 100 μm .

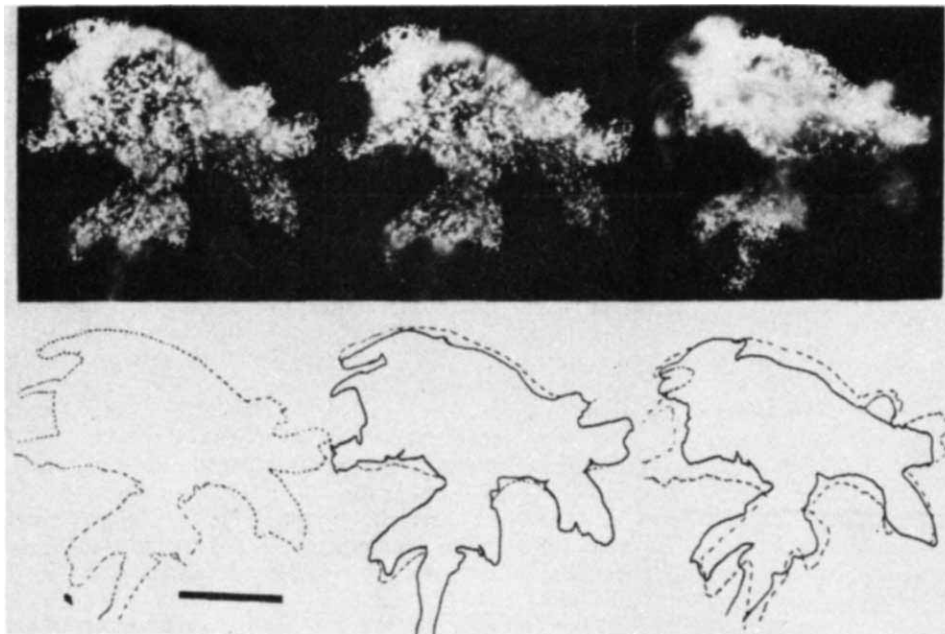


Fig. 2 Sequence illustrating amoeboid model contraction, displacement of endoplasm, rupture of plasmalemma (ectoplasm) and expulsion of some endoplasm. ----, Gives the beginning of contraction; —, is photograph above. The bar is 100 μ m.

In preliminary experiments, the endoplasm of glycerinated models was moved reversibly in either direction by applied external pressures at values which have been found to move amoeboid endoplasm *in vivo*^{6,8}. Pseudopodia could be collapsed or expanded by pressure depending on flow of the glycerinated endoplasm.

Numerous investigators have reported muscle-like proteins in amoeba (see refs 9–11). Some actin-like filaments may be attached to the plasmalemma⁹; whereas, myosin-like proteins do not always display a 'thick' filament component as reported in *Acanthamoeba castellanii*¹⁰. But thick filaments can have ATPase activity¹⁰. Also, thick and thin filaments have a remarkable orientation and interdigitation in the ectoplasmic tube region of *A. proteus* similar to striated muscle cells¹¹. Although thick and thin filaments are present in glycerinated *Chaos-Proteus* amoebae, these filaments, their interactions and sites during contraction-relaxation have not been determined by electron microscopic investigation. It seems, however, that sliding filaments as proposed for muscle contraction¹² constitute the basic contractile mechanism for amoeboid motion^{13,14}.

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Bilateral cortico-tectal projection from the visual cortex in the cat

THERE is anatomical and electrophysiological evidence for a heavy and well organised fibre projection from the visual cortex on the superior colliculus of the midbrain in several species^{1–4}. In the cat, areas 17, 18 and 19 of the visual cortex each project to the colliculus and the arrangement is such that a point in the cortex related to a particular part of the retina sends fibres to that part of the colliculus receiving fibres directly from the same part of the retina. The available evidence is for a cortico-tectal projection to the ipsilateral superior colliculus only. In several brains of cats with lesions involving the visual cortex of one hemisphere, fibre degeneration has now also been found in the superior colliculus of the opposite side. This observation provides an explanation for several findings made in electrophysiological studies of the representation of the visual field in the superior colliculus which have been difficult to interpret, and it should help in the interpretation of functional studies on the visual connections of the cerebral hemisphere.

Studies of the visual system of the cat have made available a number of brains with lesions of varied size in the visual cortex and in the superior colliculus. The cats were anaesthetised with intraperitoneal Nembutal and, with aseptic precautions, lesions were made under direct vision in the visual cortex of one hemisphere or electrolytically in the superior colliculus of one side by a horizontal stereotaxic approach through the cerebellum. After survival periods of 4–6 d the animals were perfused with saline and 10% formalin and the brains were immersed for a further period in the same fixative. Frozen sections were cut in the coronal plane and a 1:10 series of sections stained with the Fink-Heimer or Wiitanen methods^{5,6}.

After a large lesion of the visual cortex with damage to or undercutting of areas 17, 18 and 19, there is fibre and terminal degeneration in the superficial four layers of most of the extent of the superior colliculus on the same side of the brain (Fig. 1a and b). The features of this degeneration are similar to previous descriptions based on the Nauta-Gygax method. There are horizontally disposed fibre fragments and fine granular degeneration in the stratum zonale; in the stratum griseum superficiale the degeneration is very dense in its deep half and much less in its superficial part;

in the stratum opticum the fibre fragments are coarser with less terminal degeneration, whereas in the stratum griseum intermediale the intensity of the degeneration is less and diminishes progressively towards its deep margin. On the contralateral side there is degeneration in the same superficial layers over a restricted area close to the medial and lateral margins. It is present over approximately the rostral half of the lateral margin, affects rather less of the medial margin and some continues round the rostral end of the colliculus (Fig. 1b and c); this part of the superior colliculus contains the representation of the midline of the retina²⁻⁴. The intensity of the degeneration is less than that of the operated side and is most evident in the stratum opticum and stratum griseum superficiale. Degeneration on the contralateral side is also found after smaller lesions of the visual cortex, and when the lateral half only of the ipsilateral colliculus contains degeneration, as after damage of the anterior half of the visual cortex, the degeneration on the contralateral side is found only at the lateral margin. Even with relatively small lesions at the boundary of areas 17 and 18 a few fibre fragments are found in the contralateral colliculus and always at a site corresponding to that on the operated side.

In three Siamese cats with lesions in the visual cortex degeneration is also present close to the margins of the anterior half of the contralateral superior colliculus as well as on the ipsilateral side. In one of these cats, the lesion was virtually restricted to the cortex related to the aberrant projection of the 20° of the ipsilateral visual field⁷ (Fig. 1d).

The fibres which terminate in the contralateral colliculus probably reach that side after passing through the ipsilateral colliculus. The evidence in support of this is that no fibre degeneration has been seen in the brachium of the superior colliculus on the contralateral side, but a few degenerating fibres can be seen passing through the deeper layers of the superior colliculus on the operated side into the commissure of the superior colliculus. In addition, in several cats with

lesions restricted to the superior colliculus of one side there is severe degeneration throughout the deeper layers of the contralateral superior colliculus with less in the stratum opticum; in most of the extent of the stratum griseum superficiale there is only an occasional fragment and there is none in the stratum zonale. In those parts containing the representation of the midline of the retina, and in which the fibres from the contralateral visual cortex terminate, the distribution and intensity of the degeneration is the same as after lesions of the cortex (Fig. 1e).

The finding of the projection from the visual cortex to the superior colliculus of both sides and the fact that the fibres to the contralateral nucleus end in the site of the representation of the midline of the retina may help to explain certain electrophysiological observations. It has been found in the cat that about 40° of the ipsilateral hemifield are represented in the rostral part of the colliculus and that this representation is binocular⁸⁻¹⁰. The pathway serving the representation of the ipsilateral hemifield has not been determined but it has been suggested that it is an indirect one and not direct from the retina^{8,10}. The cells in the superficial layers of the representation of the ipsilateral hemifield had small receptive fields which were close to the midline and those in the deeper layers were large and extended further from the midline¹⁰. On the basis of these findings we suggest that the pathway for the ipsilateral hemifield representation to the superficial layers is through the projection from the contralateral visual cortex and that to the deeper layers is from the superior colliculus of the opposite side. The finding of a similar crossed cortical fibre pathway in the Siamese cat is interesting because the representation of the ipsilateral hemifield seems to be related to the opposite eye only¹⁰, and because some fibres at least of the pathway to the opposite colliculus seem to arise either in the part of the cortex related to the aberrant projection of the 20° of the visual field or in the immediately adjoining part of the visual cortex. Finally, as the superior colliculus is now known to send fibres to the lateral geniculate nucleus and other thalamic nuclei^{11,12}, the contralateral projection to the opposite superior colliculus from the visual cortex could serve as an indirect pathway from the visual cortex of one hemisphere to that of the other hemisphere even after section of the corpus callosum and other forebrain commissures.

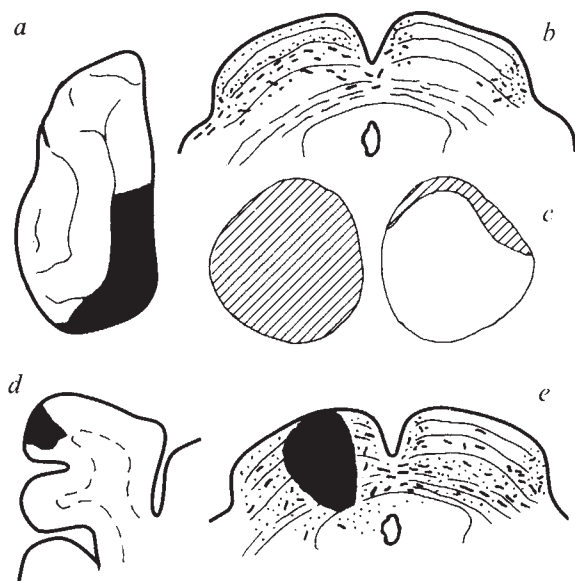
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Fig. 1 The distribution of the fibre (dashes) and terminal (dots) degeneration in the superior colliculus of the two sides of the midbrain, in a coronal section (d) and (as hatching) on an orthographic reconstruction (c), after a large lesion (solid black) of the visual cortex (a). The solid black in (d) shows the site of the lesion in the representation of the aberrant 20° of the visual field of a Siamese cat, which also resulted in fibre and terminal degeneration in the contralateral superior colliculus. The solid black in (e) shows the site of a lesion restricted to the superior colliculus of one side and the resulting degeneration in a coronal section of the midbrain.



Size accentuation in the dominant eye

CONTRARY to popular opinion, people do not use their two eyes equally. It has been found that in tasks where only one eye can be used (such as sighting down a telescope) ~65% of all observers consistently select the right eye, 32% the left and only 3% are ambicular¹⁻⁴. This preference⁵ occurs even when both eyes are open, as when one points to a distant object with the finger-tip. If one alternately closes each eye, it will be apparent that the distant target is aligned with the dominant eye but not with the other. Here we report investigations into whether

there is a difference in the apparent size of stimuli presented to the dominant and non-dominant eye. We found that $\sim 2/3$ of our subjects see the object as being bigger with their dominant eye.

A sample of 45 observers was pretested to verify equal acuity in the two eyes. Each was screened to determine sighting dominance using two tests. The first dominance test was the Point Test, which involves having the observer point to the experimenter's nose with both eyes open. The experimenter notes which eye is aligned with the finger. Four repetitions were administered with the observer alternating hands to control for manual dominance. The second procedure for assessing eye dominance was the ABC Test, in which the observer holds the wide end of a truncated cone up to his face and views a target through the smaller aperture while keeping both eyes open. The cone must be squeezed in order to be held open, and the eye aligned with the aperture is scored as the dominant eye. There were four repetitions of this test. These procedures have been shown to be reliable indicators of the sighting dominant eye^{1,3,5,6}. The sample was selected to contain 25 right-eyed and 20 left-eyed subjects.

Observers next viewed two white circular targets with a diameter of 10.5 cm at a distance of 40 cm through a haploscope which separated the inputs to the two eyes. They were asked to judge which of the two disks appeared 'larger' to them. This was a simple phenomenal judgment which all observers made without difficulty. Of the right-eyed subjects, 17 thought the object shown to their right eye to be larger, while of the left eyed, 13 thought objects presented to that eye the larger. In both cases, therefore, $\sim 2/3$ of the subjects thought that objects seen with their dominant eye were larger. These results are statistically reliable with a $\chi^2 = 4.90$, $P < 0.05$.

This difference is clearly psychological, rather than reflecting different magnification in the two eyes, since observers had been prescreened for equal refraction in both eyes. The reasons for these size distortions are not immediately clear. It seems likely that it may arise from image enhancement in the dominant eye or, perhaps, to differential selective attention to that image which gives rise to accentuation.

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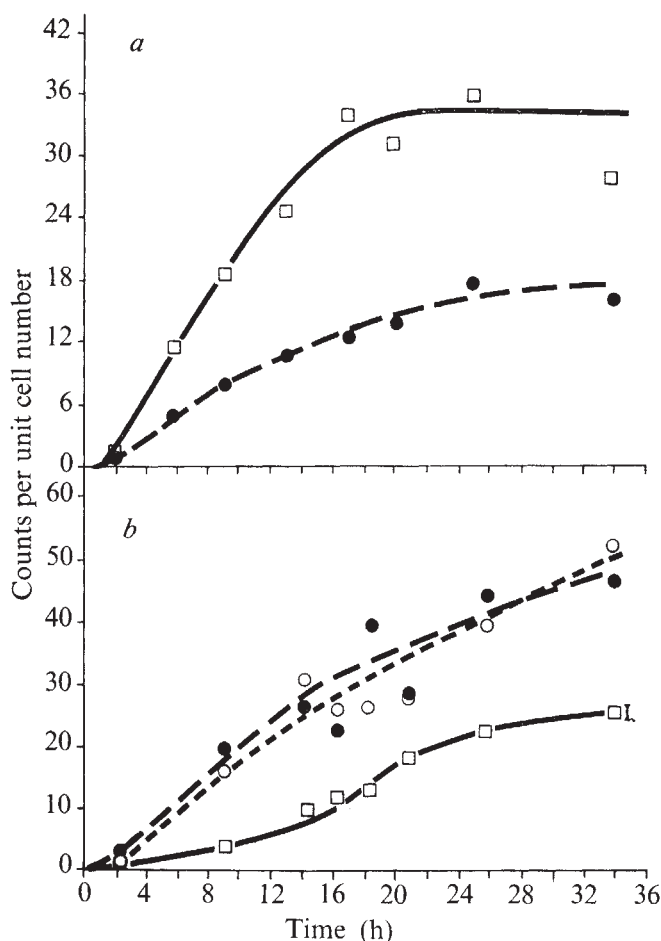
Possible role of surface Ig in non-random recirculation of small lymphocytes

THE selective extravasation of some lymph-borne immunoblasts in the gut-associated lymphoid tissue (GALT) is well established^{1–5}. It seems that immunoblasts generated in the GALT return to it, whereas those formed in peripheral somatic lymph nodes (PSLNs) go to the spleen or other lymph nodes⁶. On the other hand, for want of contrary evidence, it is assumed generally that the small lymphocytes comprising the recirculating pool recirculate more or less randomly through the various nodes in the body. T and B lymphocytes may have

different rates and microanatomical routes of recirculation through a given lymphoid organ^{7–9} but until now it has not been possible to show that recirculating lymphocytes discriminate between different lymphoid organs. By comparing the recirculation of small lymphocytes from the intestinal lymph with that of lymphocytes from the efferent lymph of PSLNs in unanaesthetized sheep, we found that each population tends to recirculate preferentially through the type of lymphoid tissue from which the collections were made.

Crossbred adult wethers or ewes were anaesthetized and polyvinyl cannulae were inserted into the intestinal lymph duct and into an efferent duct of one or two of the PSLNs (popliteal, prefemoral or prescapular). After operation the conscious sheep were placed in metabolism cages and the lymph collected quantitatively^{10–12}. Serial collections were made over periods of 8–16 h and in each experiment either a collection of intestinal lymph or a collection of efferent lymph was incubated *in vitro* with ⁵¹Cr. The washed, labelled cells were returned to the sheep through an intravenous cannula after which regular samples of each type of lymph were taken for 24–48 h. The lymphocytes were washed, counted and radioassayed and their specific radioactivities determined. Eight such experiments were performed; four in which labelled intestinal cells were reinfused and four in which efferent cells from PSLNs were used (Fig. 1). Cells from intestinal lymph tend to reappear in that lymph, whereas cells from peripheral

Fig. 1 The specific radioactivities (counts per unit cell number) of ⁵¹Cr-labelled small lymphocytes in various lymphatic ducts after the injection, at time 0, of 3×10^9 cells from intestinal lymph (a) or 2×10^9 cells from prescapular efferent lymph (b). The cells had been labelled *in vitro* with $\text{Na}_2^{51}\text{CrO}_4$ at $15 \mu\text{Ci ml}^{-1}$ for 1 h at 38°C . $\square$, Intestinal lymph; $\bullet$, prefemoral efferent lymph; $\circ$, prescapular efferent lymph. Counting rates in individual samples were usually well above 10^3 c.p.m. except for the first few hours after injection of the labelled cells.



effluent lymphatics tend to recirculate through PSLNs; this happened quite unequivocally in every experiment.

In all experiments 2% of the injected labelled cells were immunoblasts; by labelling the immunoblasts with ^{125}I -iododeoxyuridine (^{125}I UdR) it was possible to compute the amount of ^{51}Cr associated with this type of cell and rule out the possibility that their presence could bias the results. The lower graph (b) confirms our previous report¹³ that effluent cells from any one PSLN will recirculate equally well through any other PSLN. It should also be noted that there was no fall in the specific radioactivities of the lymph cells within the experimental period. This suggests a longer recirculation time for lymphocytes in sheep than in rodents¹⁴, but is in agreement with other results from sheep^{13,15}.

It is obvious that the distinction between peripheral and intestinal recirculation is by no means absolute. This may indicate that there is only a tendency for a given cell to return to its place of origin, but the results could just as well be explained by the presence of two populations; one made up of cells with a strong preference for a given pathway, and the other made up of randomly recirculating cells. The return of immunoblasts to the GALT is possibly mediated by their immunoglobulin A (IgA) or a closely associated molecule^{6,14}. It seems plausible, therefore, that those small lymphocytes which recirculate preferentially through the GALT do so because of their α -associated determinants, as up to 40% of all Ig-bearing lymphocytes are believed to have α chains on their surfaces¹⁷. Thus, we suggest that the route of recirculation of B cells may be influenced by the class of their surface Ig, while the non-Ig bearing T cells recirculate randomly. Sordat *et al.*¹⁸ found significant amounts of Ig in the walls of post-capillary venules in the tonsil and although functional evidence was lacking they suggested that the presence of Ig was in some way related to lymphocyte recirculation. If these ideas are true it is possible that the number of B cells available to a given tissue may be limited to those with the corresponding Ig subclass; this could be important in determining the nature of immune responses and possibly also the metastatic pattern of some malignant lymphomata.

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Serum-dependent adhesion and cytotoxicity of cells to *Litomosoides carinii* microfilariae

SERA from infected or immunised hosts have been reported to promote adhesion of leukocytes from those hosts to a variety of parasitic helminths^{1–3}. Higashi and Chowdhury⁴ found selective adhesion of eosinophils to the infective larvae of *Wuchereria bancrofti* in the presence of serum

from filariasis patients showing microfilaraemia, lymphoedema or elephantiasis. In albino rats infected with the filarial parasite *Litomosoides carinii* a termination of microfilaraemia (onset of latent infection) is associated with the adhesion of cells to microfilariae in the pleural cavity where the adult worms reside. Cells which seem morphologically to include macrophages, lymphocytes and polymorphs can be seen attached to living as well as to disintegrating microfilariae⁷. Virtually no attachment is seen during the patent stage of the infection. Furthermore, immunosuppression of rats with latent infection by means of cortisone, cyclophosphamide anti-lymphocyte serum or whole-body irradiation can lead to the appearance of microfilaraemia in the animals^{7–9}. Because the observations suggest the occurrence of an immune reaction, perhaps cell mediated, against microfilariae we investigated the mechanism of cellular adhesion. The results reported here show that adhesion *in vitro* is mediated by a serum factor and is accompanied by a cytotoxic effect on microfilariae.

Microfilariae were isolated from heparinised blood of *L. carinii*-infected rats by sedimentation of blood cells with dextran (molecular weight 200,000–275,000). They were washed and resuspended in tissue culture medium (HEPES-buffered RPMI1640 (Gibco)), containing penicillin (100 U ml⁻¹) and streptomycin (100 μg ml⁻¹). They were then counted in a haemocytometer. Spleen cells were prepared by teasing the tissue in medium and were washed twice before use. The peritoneal cavities of normal rats were washed out with 10 ml of the medium containing heparin (10 U ml⁻¹) and the cells were cultured in ring-and-slide chambers¹⁰. The non-adherent cells were washed off after incubation for 2 h at 37 °C, leaving cultures of peritoneal macrophages. The sera used were obtained from normal rats, from rats with patent infection and from rats with latent infection (samples from eight rats in each group) and from rats and rabbits hyperimmunised with homogenates of adult parasites in Freund's complete adjuvant (samples from two rats and two rabbits).

For adhesion experiments the reaction mixtures contained microfilariae and spleen cells at a ratio of 1:500–1:2000 in a volume of 0.5 ml of medium with 20% rat serum. They were incubated at 37 °C for 16 h with occasional shaking. A drop of the mixture was examined microscopically and the percentage of microfilariae having cells attached calculated. Adhesion occurred only when the mixture contained serum from rats with latent infection. Some typical results are presented in Table 1, which shows that spleen cells from rats with latent infection can become attached only in the presence of latent rat serum and that cells from mice and guinea pigs, but not man, can become attached, although to a lesser degree. Other experiments showed that adhesion was appreciable at 6 h and had reached a maximum by 16 h incubation. In general, adhesion was not seen in mixtures containing less than 10% latent rat serum. In

Table 1 Adhesion of spleen cells to microfilariae

Source of cells	Source of serum	% Microfilariae with spleen cells attached
Normal rat	Latent rat	80
	Normal rat	2
	Infected rat	4
	Immunised rat	0
	Immunised rabbit	0
Latent rat	Latent rat	75
	Normal rat	4
Mouse	Latent rat	54
Guinea pig	Latent rat	17
Human*	Latent rat	0

*Peripheral leukocytes were used in the experiments.

Table 2 Role of complement in adhesion

Experiment	Treatment of latent rat serum	% Microfilariae with spleen cells attached
1	None	92
	Heated (56 °C 30 min)*	0
	Heated+NRS†	39
2	None	78
	HSA-anti-HSA*	10
	HSA-anti-HSA+NRS†	20

*No haemolytic complement activity detectable.

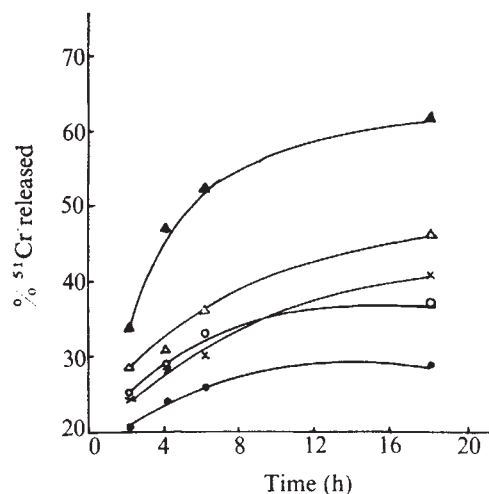
†Amount of normal rat serum (NRS) used completely lysed 10⁹ antibody-sensitised sheep red blood cells. Mixture contained 20% latent serum and 20% NRS. HSA, Human serum albumin.

other experiments spleen cells which had been passed through a glass wool column¹¹ became attached to only 15% of microfilariae in the presence of latent rat serum, whereas virtually all microfilariae become attached to peritoneal macrophages after incubation in the presence of latent rat serum.

On the assumption that immunoglobulin antibodies were required for adhesion, the role of complement was investigated. Table 2 shows that latent rat serum decomplemented by heat (56 °C, 30 min) or by an antigen-antibody precipitate failed to induce adhesion and that its capacity to induce adhesion was only partly restored by normal rat serum. The poor restoration in the second experiment may have been due to persistence of small amounts of immune complexes, which are potent inhibitors of reactions between cells and antibody-treated targets¹². These observations may indicate the operation of two mechanisms for inducing adhesion, one involving antibody only and one involving antibody plus complement.

Further experiments were carried out to determine whether lymphoid cells damaged microfilariae in the presence of latent rat serum. Microfilariae liberated from washed adult worms during incubation (37 °C, 1 h) in medium were labelled with ⁵¹Cr essentially as described by Butterworth *et al.*¹³ for schistosomulae, except that tissue culture medium containing 20% normal rat serum was used. They were then washed five times in medium and resuspended in medium containing normal rat serum. The suspension contained 3,000 microfilariae per ml, giving about 300 c.p.m. per microfilariae. They were incubated with normal spleen cells and with additional serum (to a concentration of 20%) at 37 °C. The release of ⁵¹Cr was measured at intervals. Figure 1 shows that there was a marked increase

Fig. 1 Release of ⁵¹Cr from labelled microfilariae on incubation with normal spleen cells and latent rat serum. ▲, Normal spleen cells (NC) + latent rat serum (LRS); △, NC + normal rat serum (NRS); ×, NRS; □, LRS; ●, medium.



in ⁵¹Cr release over control levels in the presence of latent rat serum and normal spleen cells.

Our earlier observations⁷⁻⁹ suggested that cell-mediated immunity may be important in the destruction of microfilariae. The experiments described here implicate both humoral and cellular factors in a collaborative reaction, which may be similar to that directed against schistosomes.

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HeLa cells secrete α subunit of glycoprotein tropic hormones

THE HeLa line, derived from a carcinoma of the cervix, was the first continuous human tissue culture line to be established *in vitro*¹, and has been one of the primary sources for the study of human cell biology. Although such studies have documented the biosynthesis of certain intracellular and membrane proteins²⁻⁴, there has been little specific characterisation of proteins secreted by these lines. The human glycoprotein tropic hormones, thyroid-stimulating hormone (HTSH), follicle-stimulating hormone (HFSH), luteinising hormone (HLH), and chorionic gonadotropin (HCG), are each composed of two subunits—an immunologically indistinguishable and probably common α subunit (' α ') and distinct β subunits which confer biological specificity on the complete molecule⁵⁻⁸. Complete hormones and free, isolated subunits are secreted by their normal cells of origin in pituitary or placenta under a variety of physiological and neoplastic circumstances⁹⁻¹¹. Furthermore, complete hormones and isolated subunits can even be secreted ectopically—that is, by tissue which does not in normal conditions produce these proteins. Since ectopic α secretion has been documented for a variety of neoplasms, both *in vivo*¹² and *in vitro*¹³, we examined the possibility that HeLa lines might also ectopically secrete the α subunit. We have found that three different HeLa strains secrete a substance immunologically indistinguishable from the α subunit, with secretion rates differing by as much as 50-fold.

In identical laboratory conditions we have grown

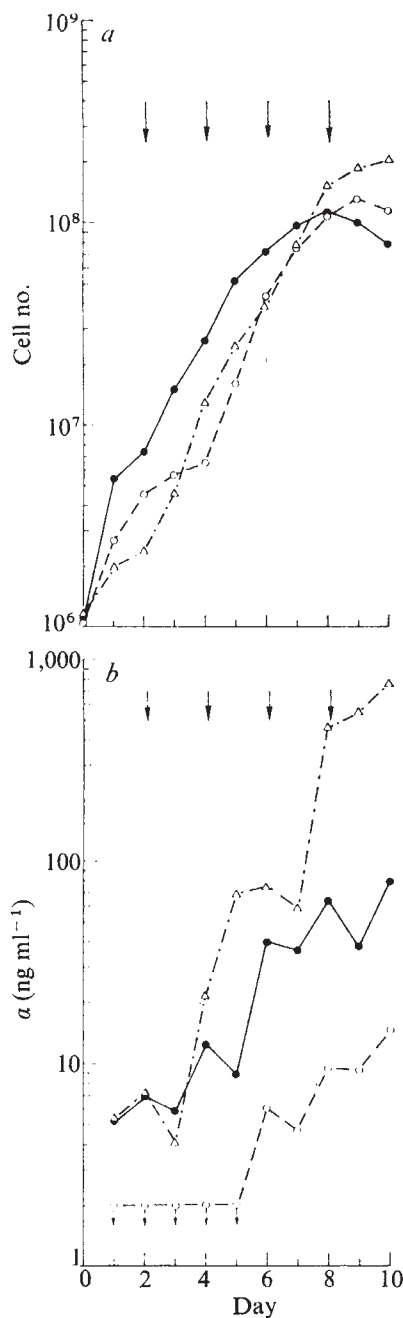


Fig. 1 Growth curves and α concentrations for the three strains: ●, HeLa CCL 2; ○, HeLa CCL 2.1; △, HeLa CCL 2.2. All cultures were established at day 0, and for each strain two cultures were used on each day of measurement. *a*, Total cell number per 75-cm 2 flask; *b*, media concentrations of α measured in the same double-antibody radioimmunoassay using HCG- α as reference standard. Arrows indicate days when the medium in each remaining flask was exchanged with fresh medium; α concentrations on those days were measured immediately before medium change. HeLa CCL 2 has also been called HeLa or HeLa(Gey), HeLa CCL 2.1 has been called HeLa 229, and HeLa CCL 2.2 has been called HeLa S $_3$.

sequential separate cultures of three HeLa strains—HeLa CCL 2, 2.1 and 2.2 (American Tissue Culture Collection)¹⁴. Each culture was grown at 37 °C in 75 cm 2 flasks in 30 ml α minimal essential medium with 10% foetal calf serum. On each day of measurement, two cultures of each strain were used. On each day, we counted cell number after trypsinisation and measured α concentration in the culture media by a specific double-antibody radioimmunoassay using

HCG- α as a reference standard¹². As the cell numbers increased, all three lines progressively accumulated α immunoreactivity in the media, although in markedly different concentrations (Fig. 1). At no time was HCG- β detected by radioimmunoassay (<0.2 ng ml $^{-1}$) in the media¹⁵. Complete glycoprotein hormones containing α subunit have small (1–3%) cross reactions in the α assay¹², and a very large amount of any of these hormones could have accounted for the α immunoreactivity. This possibility was excluded by examining the media in specific radioimmunoassays for HTSH, HLH, HFSH and HCG^{12,16,17}; all were undetectable (HTSH <1.0 μ U ml $^{-1}$, HCG <0.2 ng ml $^{-1}$, HLH <1 mIU ml $^{-1}$, HFSH <1.5 mIU ml $^{-1}$). Control medium not exposed to HeLa cells had undetectable amounts of α (<2 ng ml $^{-1}$). Media exposed to HeLa cells had no effect on 125 I-HCG- α tracer integrity, since such tracer was still fully immunoprecipitable by excess antibody to HCG- α . The peak secretory rates of α by HeLa 2.1 was 0.1, by HeLa 2 was 1.0 and by HeLa 2.2 was 5.0 pmol per 10^6 cells per day.

The ectopic secretion of α by cell lines derived from a carcinoma of the cervix is of interest. Whether the original tumour secreted α *in vivo* is not known. Certain tumours and cultures are known, however, to secrete progressively more α subunit *in vitro* with time⁹. Indeed, the K $_1$ clonal strain of the ChaGo bronchogenic carcinoma cell line secretes 290 pmol α per 10^6 cells per day, substantially more than the wild type¹⁸.

Serial dilutions of alpha in the HeLa tissue culture medium exactly paralleled standard curve dilutions of HCG- α in the radioimmunoassay, indicating virtual immunological identity. The gel chromatographic behaviour of HeLa- α was also studied (Fig. 2). Medium from a confluent culture of HeLa 2.2 was cochromatographed on Sephadex G-100 with 131 I-HCG- α as an internal marker. This procedure discriminates between the glycoprotein hormones and their subunits, and on this column the α subunits of HTSH, HFSH, HLH and HCG all elute identically¹⁹. The HeLa- α eluted in the α subunit region, again clearly differentiating it from the complete glycoprotein hormones. Although the elution pattern of HeLa- α is similar to that of HCG- α , HeLa- α does have a slightly higher apparent molecular weight. In this respect, HeLa- α is similar to the α produced by a gastric carcinoid *in vivo* and a bronchogenic carcinoma *in vitro*¹⁹. The very similarity in α produced by these diverse neoplasms raises the possibility, among several other explanations, that tumour α may be a precursor of normal α (ref. 19).

All three strains of HeLa studied secreted α ; in fact two different cultures of HeLa 2.2 obtained from different sources (American Type Culture Collection and Flow Laboratories) had almost identical α -secretion rates. It has been suggested that many human tissue culture lines have been overgrown and replaced by HeLa cells^{20–22}. Although α secretion is not exclusive for HeLa—certain cell lines such as ChaGo, which are not HeLa by karyologic criteria¹⁸, secrete α —the presence of α secretion by a human cell line should prompt careful examination for known karyologic and enzymatic HeLa markers. For example, we have found significant quantities of α (14 ng ml $^{-1}$) in the medium of HBT $_3$, a line originally derived from a breast cancer which has been found to have karyologic HeLa markers²². On the other hand, it is not known whether the absence of α secretion rules out HeLa. Three different HeLa strains, including two different sources of HeLa 2.2, secreted α , but in the case of HeLa 2.1 we could only detect α at high cell density after a number of days. We do not know, however, whether all HeLa strains secrete α even at high density after many days.

It has been reported that another HeLa strain, HeLa $_{d5}$ —a variant of HeLa CCL 2.2—secretes very small amounts (0.0024 ng per 10^6 cells per day) of material which has activity in an HCG- β immunoassay²³. This basal secretion

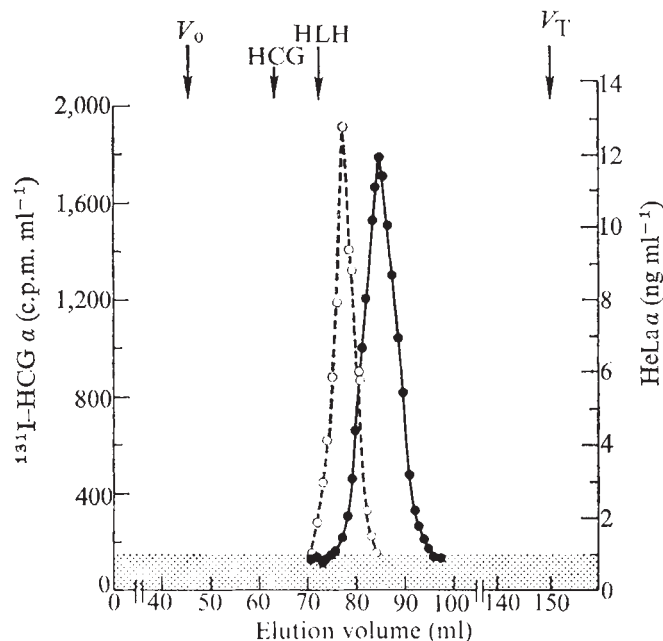


Fig. 2 Gel chromatography of ^{125}I -labelled α subunit of chorionic gonadotropin (^{125}I -HCG- α) added to medium from a confluent culture of HeLa CCL 2.2. The Sephadex G-100 column (1.5×90 cm) was equilibrated at 4°C and eluted with 0.15 M NaCl, 0.01 M Na phosphate, pH 7.4, at a flow rate of 5 ml h^{-1} ; 1-ml fractions were counted for ^{125}I radioactivity (●) and measured for α immunoactivity (○) by a ^{125}I radioimmunoassay. The shaded area is below the detection limits of the radioimmunoassay. The void volume (V_0) and the total volume (V_T) were determined in the same chromatogram. In separate experiments HCG and HLH migrated with elution volumes as indicated by the arrows. The column was calibrated with five standard proteins (apoferritin, gammaglobulin, ovalbumin, chymotrypsinogen A, and ribonuclease). HeLa- α had an apparent molecular weight of $\sim 33,000$, whereas HCG- α had an apparent molecular weight of $\sim 24,000$. The molecular weight calculated from the chemical composition of purified HCG- α ($15,000$) was used to calculate the secretion rates in the text. Differences between the apparent molecular weight of α and that calculated from chemical composition have been discussed elsewhere¹⁸.

rate is four orders of magnitude less than the α secretion ($75\text{ ng per } 10^6\text{ cells per day}$) by HeLa CCL 2.2 reported here. Although this reported HCG- β immunoactivity (HCG and/or HCG- β) may to some extent be accounted for by cross reaction with large excess of free α , it has been observed that certain ChaGo clones which secrete large amounts of free α also secrete small amounts of biologically active HCG¹⁴.

We do not yet know what relation α secretion by these HeLa strains has to their different karyotypes or even to intracellular production of other HeLa proteins such as placental alkaline phosphatase. The fact that three HeLa strains derived from a carcinoma of the cervix have widely different secretion rates of α suggests that they may be useful in identifying the factors controlling the production of this protein, which is normally repressed in cervical cells.

A number of diverse neoplasms have been shown to secrete the α subunit ectopically *in vivo* and/or *in vitro*; these include cancers of lung¹³, stomach¹², pancreas¹², and now uterine cervix. Studies of α secretion should be extended to other human cell lines and, when assays become available, to cell lines from other species. Perhaps the α subunit will turn out to be a relatively easily derepressed and 'primitive' protein.

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Interference between anti-HLA antibodies and chlorpromazine

It has been suggested that the effects of chlorpromazine in man and animals are genetically influenced^{1,2}. In mice, genetic control of sensitivity to this drug seems to reside in two loci, the more important of which lies on chromosome 9 at about the region which controls the histocompatibility system H2 (ref. 3). We have investigated whether the presence or absence of some alleles of the HLA system—analogueous to the H2 system of mouse—correlate with individual sensitivity to the drug. We evaluated the clinical responses to chlorpromazine of 33 chronic schizophrenic subjects who had been HLA typed during previous investigations^{4,5}. There was a highly significant positive response to chlorpromazine in patients with the antigen HLA-A1 of the HLA-A locus (unpublished results of E.S., L. Bellodi and E. Sacchetti). A possible explanation of the correlation is that the presence of HLA-A1 on the cell membrane makes chlorpromazine bind more readily to the cell. As chlorpromazine binds to lymphocyte membranes⁶, we investigated whether or not the binding of the drug could interfere with the specific binding of anti-HLA antibodies, particularly anti-HLA-A1 antisera. Furthermore, as chlorpromazine binds to the β -adrenergic cell receptors, which are also present on peripheral blood lymphocytes⁷, we also used the β -adrenergic mediators dopamine (DA) and noradrenaline (NA).

Five healthy volunteers were selected on the basis of their HLA types from our HLA reference panel. Three were HLA-A1 positive and two were negative for the

Table 1 Effect of chlorpromazine, DA and NA on absorption of two anti-HLA-A1 sera and serum MI P1 (anti-HLA-B₁₃,B₁₅) by human peripheral blood lymphocytes

	MI P1					MI P42					Morrison				
	1,9,7,12	1,5,8	1,12,8	2,5,12	9,12	1,9,7,12	1,5,8	1,12,8	2,5,12	9,12	1,9,7,12	1,5,8	1,12,8	2,5,12	9,12
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Original cytotoxic titre	1/8	—	1/8	1/16	1/8	1/16	1/8	1/16	—	—	1/16	1/16	1/16	—	1/4
Cytotoxic titre after absorption with fresh lymphocytes	—	—	—	—	—	1/2	—	—	—	—	—	—	—	—	—
Cytotoxic titre after absorption with lymphocytes preincubated with															
Chlorpromazine (0.4×10^{-4} M)	1/8	—	1/8	1/16	1/8	1/16	1/8	1/16	—	—	1/8	1/8	1/16	—	—
DA (0.8×10^{-4} M)	1/8	—	1/8	1/16	1/8	1/4	1/8	1/8	—	—	1/8	1/8	1/8	—	—
NA (0.8×10^{-4} M)	1/8	—	1/8	1/16	1/8	1/4	1/4	1/8	—	—	1/4	1/8	1/8	—	—

Results are expressed as the serum dilution giving a cytotoxicity > 80%.

same antigen. Each subject was typed many times in different typing sessions and tested with the antisera used in this work. The types were: G.C.: HLA-A1, A9, B7, B12; L.B.: HLA-A1, B8, B12; L.M.: HLA-A1, B5, B8; B.P.: HLA-A9, B12; R.S.: HLA-A2, B5, B12. Thirteen anti-HLA antisera were selected from sera supplied from the NIH bank (Bethesda) and from cytotoxic sera from multiparous women screened in our laboratory for their specificity and compared with highly selected antisera from the Institute of Medical Genetics of the University of Torino. The sera, with their corresponding specificities were: Morrison: anti-HLA-A1, A9, B8; MI P42: anti-HLA-A1, AW25; MI P80: anti-HLA-B8, AW32+; MI P106: anti-HLA-A2; MI P138: anti-HLA-A2; MI Pt1 18/3: anti-HLA-A9; MI P145: anti-HLA-A9; D66622IX: anti-HLA-B5; MI P71: anti-HLA-B5+; MI P55: anti-HLA-B7; MI P128: anti-HLA-B7; MI P1: anti-HLA-B12, B13; MI P109: anti-HLA-B12+.

Each antiserum was tested for its cytotoxic titre against lymphocytes bearing the corresponding HLA specificity. Lymphocytes (6×10^6)—obtained from heparinised peripheral blood by flotation on Lymphoprep⁸—were incubated in Hanks' solution (buffered at pH 7.2 by adding isotonic bicarbonate solution) containing chlorpromazine or DA or NA at a concentration of 1.5×10^{-4} M and rocked at 2 °C for 1 h. Afterwards, tubes were centrifuged at 0 °C for 15 min at 600g, the supernatant layers were discarded and the cells washed once with cold Hanks' solution. Then, the washed lymphocytes (6×10^6) were resuspended in 0.3 ml of HLA cytotoxic antisera and incubated again at 2 °C for 1 h. Controls were carried out by incubating the same amount of cytotoxic antisera with 6×10^6 lymphocytes which had not been preincubated with the drugs. Afterwards tubes were centrifuged at 600g for 15 min at 0 °C and the supernatant layers, after a further centrifugation at 2,000g, were tested for their cytotoxic titre on fresh lymphocytes from the donors used for the absorption study.

We found no difference between the cytotoxic titres of antisera absorbed by control lymphocytes and by lymphocytes preincubated with drugs, except for the anti-HLA-A1 sera MI P42 and Morrison when tested with HLA-A1 positive cells (Table 1). Morrison serum showed no variation when it identified the antigen HLA-A9 on HLA-A1 negative cells (donor B.P.).

We also made a dose-inhibition study to determine the lowest concentrations of the drugs we were investigating, which were able to cause the greatest inhibitions of anti-HLA-A1 antisera.

We found that chlorpromazine was active at 0.4×10^{-4} , whereas DA and NA had their greatest effects at 0.8×10^{-4} M. At a higher concentration of chlorpromazine (0.6×10^{-3} M) we observed slight inhibition of two other antisera not detecting HLA-A1—sera MI P109 and MI P71. At the same concentration, DA and NA were ineffective.

Our data suggest specific binding of chlorpromazine to the antigen HLA-A1 of the first HLA locus which interferes with the specific binding of the anti-HLA-A1 immune sera. DA and NA also seem to bind to the HLA-A1 antigen, but perhaps with a lower affinity than chlorpromazine, as greater concentrations were needed to exert less inhibition than that due to chlorpromazine.

We think two interpretations of our results are possible. First, the HLA antigens could be structurally linked to the β -adrenergic lymphocyte receptors in the cell membrane grid. HLA-A1 might modify the quaternary structure of these receptors, making it easier for chlorpromazine to bind it and making possible a reciprocal steric inhibition of anti-HLA-A1 antiserum and chlorpromazine or DA and NA. The other HLA antigens, less intimately connected with the β -adrenergic receptors, show no such reciprocal interference. There is another possibility as many cell membrane structures are originally derived from a common ancestor. For example, all the structures governed by the HLA region are phylogenetically related^{9,10}. Moreover, the structural analogies between the immunoglobulins, the HLA antigens and β_2 -microglobulin have also suggested a common evolutionary origin for these molecules¹².

Thus we think it possible also that β -adrenergic receptors and HLA antigens descend from a single precursor and that HLA-A1 is the antigen which is less divergent from this common original structure and from the cell β -adrenergic receptor than the other HLA specificities. For this reason it would be interesting to investigate the relationships between such receptors and those HLA antigens which cross react with HLA-A1.

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Chromosome rotation and formation of synapsis

ALTHOUGH the rotation and oscillation of the nuclei of cultured spermatocytes have been known for some years^{1,2}, no correlations of this phenomenon with the stages of mitosis or meiosis, or with the biochemical or cytogenetic events during germ-cell development have been made. Using a new technique for the rapid separation of segments of rat seminiferous tubules containing accurately known cellular associations and stages of meiotic prophase³ we have been able to correlate the rotation of living spermatocyte nuclei with synapsis during rat spermatogenesis.

Immediately after removing the testes from the animal, the seminiferous tubules were removed and transilluminated under a stereomicroscope. Cells representing different stages of meiotic prophase were selected for analyses with time lapse phase-contrast cinemicrography⁴. Figure 1 shows a phase-contrast photomicrograph of early zygotene spermatocytes developing synchronously at stage XII of rat spermatogenesis. The chromosomes of cell *c* are not sharp in the figure, indicating chromosome movement during the 4-s exposure time. That these structures really are chromosomes, was shown by Pleshkewych and Levine⁵ using electron microscopic preparations of mouse primary spermatocytes, previously analysed by phase-contrast microscopy.

At the beginning of meiotic prophase at stages IX–X (ref. 6) no movements were observed in the chromosomes of the leptotene spermatocytes. In late leptotene spermatocytes, at stage XI, some nuclei were seen rotating, and an oscillatory movement of the chromosomes was common. At the next stage

Fig. 1 Phase-contrast photomicrograph of early zygotene spermatocytes developing synchronously at stage XII of rat spermatogenesis. Seminiferous tubular fragments containing accurately identified cellular associations and stages of meiotic prophase were separated using a variation in the transillumination pattern of freshly isolated unstained seminiferous tubules as a marker for different stages of spermatogenesis³. The cells were squeezed to form a monolayer between object and cover glass slides, and observations were made under phase-contrast optics. The rotational pattern of the cells *b–f* are analysed in corresponding tracings in Fig. 2. Owing to the 4-s exposure time, the chromosomes of cell *c* have moved and are unsharp in the figure. Note the size difference of the late pachytene spermatocytes.

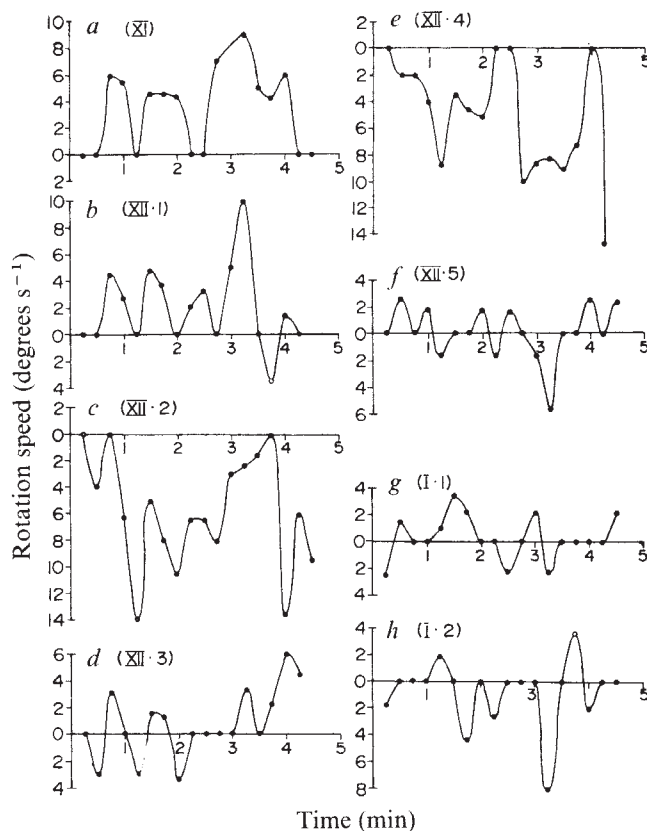
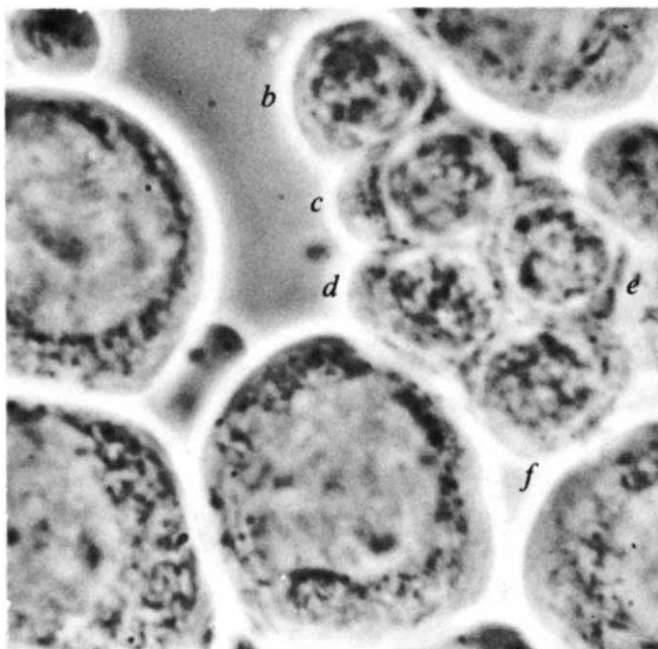


Fig. 2 Analysis of the rotating movements of the chromosomes of rat primary spermatocytes at different stages of development. Time-lapse cinemicrographs were taken at a speed of 2 frames s^{-1} with a Beaulieu 4008 ZM camera using super-8 film. The film was analysed using a viewer, which makes it possible to trace the interesting frames and to measure the time intervals. All observations were made within 4 h of killing the animal and 3.5 h of sealing the specimen chamber. The rotation speed is indicated as degrees s^{-1} at the ordinate, clockwise direction above the abscissa and counterclockwise direction below it. The observation time (min) is indicated on the abscissa. The movement is not even, and most cells change the rotation direction several times during the observation. The maximum speed (cell *e*) is 14.6 degrees s^{-1} . Tracings *b–f* are for cells belonging to the same synchronously developing group of cells depicted in Fig. 1.

(XII) the early zygotene spermatocytes showed the most active rotations and oscillations of their chromosomes. The movements then gradually slowed down towards the end of zygotene stage (XIII–XIV) and ended during the early pachytene stage (before stage III). Some oscillatory movements of the chromosomes were seen up to stage VI, but mid- and late-pachytene spermatocytes (stages VII–XII) showed no chromosomal movements.

In individual cells, the speed and direction of the rotational movement varied considerably. Figure 2 shows an analysis of cells from early zygotene (stages XI and XII) and early pachytene (stage I) primary spermatocytes. The chromosomes were followed on time-lapse film and traced every 15 s, and the rotation angle was measured. The clockwise rotation speed (degrees s^{-1}) is seen above the abscissa and the counterclockwise rotation speed below it. The cells were followed during 4.5 min (abscissa). The speed of the nuclear rotation was not even in any cell, and in most cases the direction of the movement changed during the observation time. In addition, the chromosomes seemed to move around several axes in numerous different planes. This is not shown in the tracings. Even in synchronously developing clones of early zygotene spermatocytes (Fig. 1) the rotation speed and direction vary considerably between individual cells (Fig. 2, *b–f*).

It is generally known that during zygonema (stages XII–XIV in rat spermatogenesis) the homologous sets of sister chromatid

pairs begin to come together and associate with one another. The result is a precise point-for-point alignment, synapsis. The fully developed apparatus, the synaptinimal complex, is completed during pachynema. The rotational movements of the chromosomes seem to correlate to the formation of synapsis, so that in zygonema, where the synapsis is formed, the chromosomal movements are the most rapid. In early pachynema when the synapsis is completed there is still some movement of the chromosomes, but when the synaptinimal complex is fully developed the movements of the chromosomes cease completely. The movements of the chromosomes may be helpful in the physical mutual recognition of the specific parts of chromosomes, leading to the formation of synapsis between homologous chromosomes.

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Effect of nerve growth factor on synaptic depression after axotomy

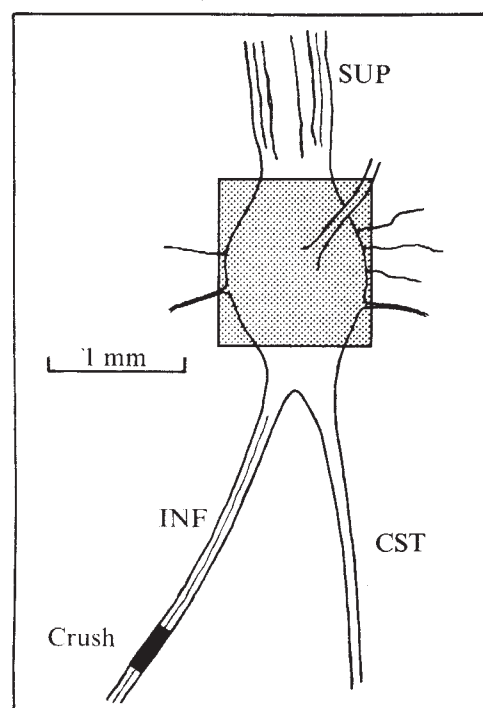
INTERRUPTION of the axon of a mammalian nerve cell leads to severe depression of synaptic transmission to the affected neurone within a few days, due primarily to loss of synaptic contacts^{1–4}. In the superior cervical ganglion of the guinea pig, recovery of synaptic contacts on many neurones occurs if axon regeneration is allowed, but generally does not take place after axon ligation⁴. Furthermore, application of colchicine to postganglionic nerves causes synaptic loss in the absence of mechanical axon interruption⁵ (see also ref. 6). These findings indicate that axonal extension to the periphery in some way provides the parent neurone with a 'trophic' signal which is instrumental in maintaining preganglionic synaptic contacts. In the sympathetic nervous system the protein nerve growth factor (NGF) has a number of effects on the metabolism and morphology of developing neurones whose survival is ultimately dependent on the presence of this agent⁷. One mechanism by which NGF affects neurones is an interaction with specific surface receptors on cell bodies⁸. More recent experiments have supported the suggestion⁹ that NGF may also act as a messenger from the effector organ to innervating sympathetic neurones: NGF is selectively taken up by sympathetic endings¹⁰, can be retrogradely transported to the neuronal somata^{10,11}, and has been shown to prevent some of the biochemical changes that follow axotomy in young animals^{12,13}. Thus a natural question to ask is whether NGF might be involved in the regulation of synapses on mature ganglion cells. We report here that exogenous NGF can, to a large extent, prevent the synaptic depression seen in adult sympathetic ganglion cells after interruption of their axons.

Ten adult guinea pigs (200–400 g) were anaesthetised with pentobarbitone and both superior cervical ganglia exposed in aseptic conditions. On the right side, the inferior postganglionic nerve was crushed 2–3 mm from the ganglion; at the same time a silicone rubber (Silastic, Dow Corning) pellet measuring approximately $1.2 \times 1 \times 0.5$ mm and containing 100–500 µg of purified 2.5S mouse salivary gland NGF¹⁴

(from Dr R. A. Bradshaw) was implanted in contact with the ventral surface of the ganglion (Fig. 1). Pellets were prepared by mixing NGF with the silicone rubber before curing. On the left side, the inferior branch was crushed, but no pellet was implanted. In a further series of seven control animals, a silicone rubber pellet without NGF was implanted at the time of crushing the inferior postganglionic branch. After 4–7 d, the interval after which postaxotomy synaptic depression is maximal⁴, ganglia were removed and studied by means of intracellular recording. Neurones on the dorsal surface of the ganglion were impaled with glass microelectrodes, and excitatory postsynaptic potentials (e.p.s.p.s) were measured in response to supramaximal stimulation of the preganglionic sympathetic trunk. Axons of individual neurones were determined to emerge in the crushed inferior branch, or the intact superior branch, by antidromic stimulation. The methods of impalement, stimulation, and e.p.s.p. measurement were as described previously⁴.

In the animals in which a control pellet without NGF had been implanted, the amplitude distribution of e.p.s.p.s in neurones whose axons had been crushed (Fig. 2b) was shifted towards lower values, compared with the synaptic responses of normal neurones (Fig. 2a). This degree of synaptic depression is similar to that after crushing alone^{4,5}. Thus, the presence of the control pellet had no effect on the course of postaxotomy synaptic depression. In each of 10 animals in which the implanted pellet contained NGF, however, the reduction in e.p.s.p. amplitude was curtailed; the synaptic responses elicited in most neurones whose axons had been crushed in treated ganglia fell within the normal range, whereas relatively few showed severe depression of synaptic transmission (Fig. 2c). In the course of studying NGF-treated ganglia, many nerve cells were impaled whose axons emerged in the intact superior branch (Fig. 1). Synaptic responses in these neurones were of normal amplitude (20–44 mV) suggesting that exogenous NGF has little effect on normal synaptic transmission. The input resistances, resting potentials, and action potential amplitudes were similar in treated and untreated control neurones whose

Fig. 1 Diagram of operative procedure. Ventral view of right superior cervical ganglion. CST, Cervical sympathetic trunk; INF, inferior postganglionic branch; SUP, superior postganglionic branch. Stippled area shows approximate size and location of silicone rubber pellet impregnated with NGF.



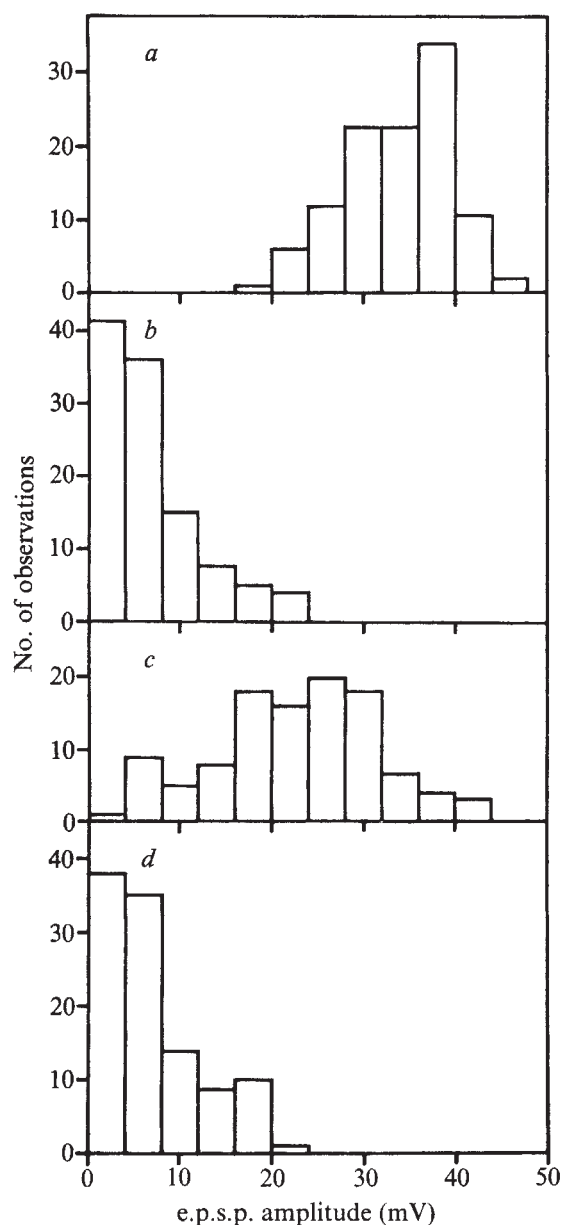


Fig. 2 Ability of exogenous NGF to curtail synaptic depression after axotomy. See text for detailed explanation. *a*, Responses of normal neurones replotted from previous work¹ (number of neurones impaled $n=112$); *b*, Axotomy + control pellet ($n=109$); *c*, axotomy + NGF pellet ($n=109$); *d*, axotomy + NGF pellet, contralateral ganglion ($n=107$).

axons had been crushed; values for these parameters were in the same range as those reported previously^{4,5}.

In ganglia contralateral to the site of the NGF-containing pellet, neurones whose axons emerged in the crushed inferior branch gave synaptic responses that were as depressed as those in animals without exogenous NGF (Fig. 2*d*; compare with Fig. 2*b*). This result indicates that the sparing effect of NGF is a local one.

Exogenous NGF also prevents at least one other electrophysiological sign of axotomy in sympathetic neurones, the development of regenerative responses in dendrites^{4,5} (see also refs 15 and 16). Rapidly rising local responses identical to those described previously^{4,5} were seen superimposed on sub-threshold e.p.s.p.s in 27% of axotomised neurones impaled in ganglia treated with a silicone pellet containing no NGF, and in 28% of axotomised neurones in ganglia contralateral to those treated with exogenous NGF. Such potentials

were observed in only 1 of 109 axotomised neurones impaled in the NGF-treated ganglia.

The rate of NGF release from the impregnated pellets was determined by incubation for 24 h in 1 ml of sterile saline either before implantation, or after removal from the animal. The incubation medium was then assayed by the chick dorsal root ganglion method¹⁷⁻¹⁹. Pellets tested before implantation released about 1,000 biological units (BU) per ml incubation fluid per 24 h, whereas pellets which had been implanted 4-7 d before testing released 10-100 BU per ml per 24 h (1 BU corresponds to about 10^{-2} μ g). Although showing that NGF is released in relatively large amounts from the pellets, these results do not provide any information about the effective concentration of NGF in the vicinity of the neurones studied.

The ability of exogenous NGF to reduce markedly synaptic depression after axotomy provides the first example of an electrophysiological effect of NGF on mammalian sympathetic neurones. We do not yet know whether the exogenous NGF acts at receptors on the surface of the cell body or intracellularly after retrograde axonal transport from the site of injury. Our results suggest, however, that NGF may normally regulate synaptic function in mature sympathetic ganglia.

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Brain immunoreactive gonadotropin-releasing hormone in Huntington's chorea and in non-choreic subjects

THERE is evidence which suggests that the release of gonadotropin-releasing hormone (GRH) is modulated by dopaminergic neurones¹⁻³. Dopamine receptors seem to be hyper-responsive in Huntington's chorea^{4,5}, and consequently the synthesis and secretion of GRH may be altered in this condition. For this reason we have compared the concentration of immunoreactive GRH in hypothalamic tissues taken *post mortem* from choreic and non-choreic ('control') subjects. The distribution of GRH in the human brain, and changes in concentration which occur with age have also been examined.

The collection of brain tissue was carried out at Cambridge as described previously⁶. At autopsy the median eminence was removed by making a horizontal incision at the edge of the

Table 1 Mean ($\pm$ s.e.m.) concentrations of GRH (pg mg^{-1}) in median eminence and hypothalamus

	Male		Female	
	Median eminence	Hypothalamus	Median eminence	Hypothalamus
Huntington's chorea	558 $\pm$ 173 (6)	18 (7)	1,231 $\pm$ 410 (9)	26 (6)
Age range	47–71	46–72	46–74	52–72
Controls	675 $\pm$ 131 (28)	27 (23)	314 $\pm$ 84 (11)	27 (15)
Age range	17–78	29–84	39–88	25–88

Numbers in parentheses indicate numbers of specimens. s.e.m. are not given for concentrations in the hypothalamus since some values in all groups were undetectable.

vascular plexus which covers this area. The remainder of the hypothalamus (here termed 'hypothalamus') was removed as indicated in Fig. 1, the dissection being completed by vertical sagittal cuts 0.2 cm on either side of the midline. The hypothalamus of three control males (aged 29, 45 and 65 yr) was subdivided into blocks containing the preoptic, suprachiasmatic, ventromedial, dorsomedial and mammillary nuclei, respectively (Fig. 1). Frontal cerebral cortex from Brodmann's areas 9–12 of seven controls and three choreics was also taken. The tissues were stored on liquid nitrogen, and transported on dry ice to Oxford where they were weighed and homogenised (15 strokes using a Potter–Elvehjem-type homogeniser) in 0.1 N HCl at 4 °C. The homogenates were stored at –25 °C. The concentration of GRH in the homogenates was determined by a double-antibody radioimmunoassay^{7,8} which is sensitive to 2–3 pg synthetic GRH per tube. The homogenates were thawed and neutralised immediately before assay, and an aliquot containing the equivalent of 0.4–0.5 mg tissue (or less at higher concentrations) was added to each of two (duplicate) assay tubes.

The slopes of the inhibition of binding curves produced by serial dilutions of the homogenates were similar to that of synthetic GRH which was used as a standard (Fig. 2), suggesting that GRH in human median eminence is immunologically

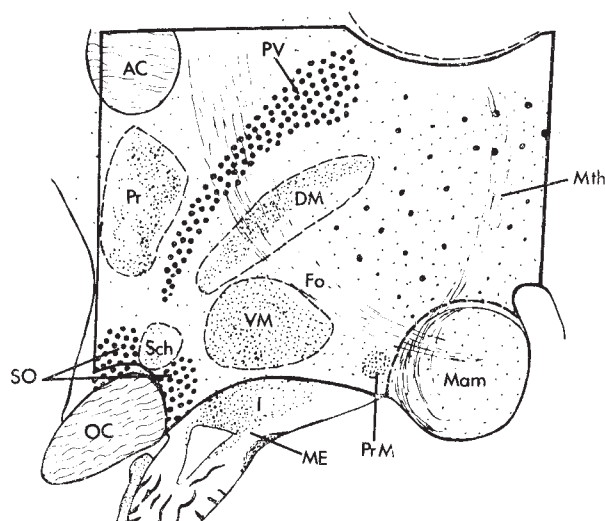
similar to that found in the median eminence of the rhesus monkey, pig, sheep and rat^{7,8}. Although the median eminence–pituitary stalk region had the greatest concentration of GRH, relatively large amounts of the hormone were also present in the preoptic nuclei (Fig. 1). In one specimen from a 45-yr old male a small block of tissue was also taken from just in front of the anterior commissure. The concentration of GRH in this tissue was 55 pg mg^{-1} . The concentration of GRH in the frontal cortical tissue could not be detected ($< 4.3 \text{ pg mg}^{-1}$) in all but one specimen from a choreic patient in which it was 16 pg mg^{-1} . In this patient, the concentration of GRH in the hypothalamus–median eminence was relatively high, 3,025 pg mg^{-1} .

The mean concentration of GRH in the median eminence of the female choreics was significantly greater ($P < 0.05$) than that in the female controls (Table 1). There was, however, no significant difference between the mean concentrations of GRH in the median eminence tissues from the choreic and control males. The concentration of GRH in the blocks of hypothalamic tissue was relatively small. In the control males the mean concentration ($\pm$ s.e.m.) of GRH in the median eminence varied with age and was 1,315 $\pm$ 467 ($n=6$), 535 $\pm$ 225 ($n=5$) and 490 $\pm$ 91 ($n=17$) pg mg^{-1} in the age ranges 17–40, 41–60 and 61–78 yr, respectively. The difference between the mean concentrations in the age groups 17–40 and 61–78 yr was significant at $P < 0.02$.

The median eminence concentrations of GRH are similar to those in rat hypothalamus (including median eminence) taken immediately before (under anaesthesia) or after death (S.A.C. and G.F., unpublished). As in the human, the greatest concentration of GRH in the rat (and guinea pig) is found in the region of the median eminence, and most but not all workers have found GRH also in the rostral hypothalamus^{8–10}. The relatively high levels of GRH in the preoptic region of the human (Fig. 1) cannot be attributed to post-mortem diffusion, for, in the same specimens, the ventromedial and dorsomedial nuclei contained much smaller amounts of GRH. In the rat, the preoptic nuclei have a critical role in the causation of the preovulatory surge of luteinising hormone (LH), and may also be important for mating behaviour¹¹. Preoptic GRH is conceivably present in neurones which send axons to the primary capillaries of the hypophysial portal vessels, but the presence of GRH in this region should also be considered in the context of the recent hypothesis that GRH may act as a neurotransmitter within the central nervous system^{12,13}.

It is not clear why the concentrations of GRH in the median eminence of choreics compared with controls differed significantly in the case of females but not males. The delay between death and the freezing of tissues was similar for the male and female specimens, and preliminary studies showed that there is no appreciable change in the concentration of GRH of hypothalamic tissue maintained at 4 °C for 6 d after death. Choreics of both sexes had been subjected to similar therapeutic regimes; all had spent the terminal stage in a psychiatric institution where they received phenothiazines regularly, and three of the males and four of the females had also been given tetrabenazine. Present radioimmunoassay methods do not enable reliable estimates of the concentration of GRH in

Fig. 1 Diagram of sagittal section of the human hypothalamus (after Diepen¹⁹). The continuous lines indicate the cuts made to prepare blocks of median eminence and hypothalamus for data in Table 1. The interrupted lines indicate blocks of tissue used to determine the distribution of GRH. The mean concentrations of GRH (pg mg^{-1}) in brains from three control males were: median eminence (ME), 760 $\pm$ 175; preoptic nuclei (Pr), 119 $\pm$ 44; suprachiasmatic nuclei (Sch), 34 $\pm$ 16; ventromedial nucleus (VM), 14 $\pm$ 1; dorsomedial nucleus (DM), 12 $\pm$ 5; mammillary bodies (Mam), 68 $\pm$ 59. I, Infundibular nuclei; PrM, pre-mammillary nucleus; SO, supraoptic nucleus; PV, paraventricular nucleus; OC, optic chiasma; AC, anterior commissure; Fo, fornix; Mth, mamillothalamic tract.



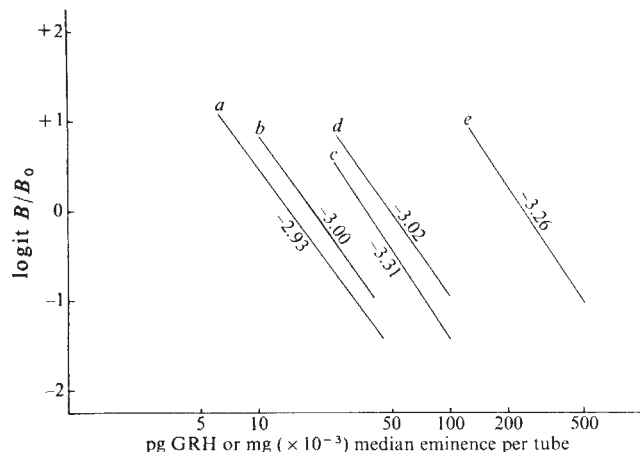


Fig. 2 Radioimmunoassay for GRH: logit* B/B_0 (percentage bound) against log dose curves of synthetic GRH (a) and serial dilutions of extracts of median eminence from choric (b, d) and control (c, e) males (c, d) and females (b, e). Lines were calculated by the method of least squares from three points (four replicates per point) in the working range 20–80% bound. Numbers adjacent to the lines are the slopes. *Logit defined by $\ln [y/(1-y)]$.

peripheral blood¹², and it was difficult to obtain reliable information regarding the pre-mortem menstrual or sexual history of these cases. The hypothalamic content of dopamine, noradrenaline and activity of glutamic acid decarboxylase in choric patients is similar to that in control subjects¹⁴.

The low concentration of GRH in the median eminence of the control females (Table 1) as well as in the older men may be due to increased release of the hormone. In the female this could not account for the marked rise in plasma gonadotropins which occurs between the ages of 45–50 yr and which is sustained at least until 75 yr (ref. 15). Although the changes are not so dramatic, the concentrations of plasma gonadotropins also increase with age in the male^{16–18}. The possibility that decreased synthesis of GRH also contributes to the lower concentrations of GRH in the median eminence of the older controls cannot be excluded.

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Aspartate and glutamate as possible transmitters of excitatory hippocampal afferents

In the rat hippocampal formation, the major excitatory inputs possess a remarkable degree of physiological and morphological plasticity. Long lasting potentiation has been reported^{1–3} and, at least in the dentate gyrus, the synaptic loss consequent on removal of one afferent is compensated by formation of additional synapses by intact afferents^{4,5}. These phenomena must involve profound alterations of synaptic biochemistry, but the requisite biochemical studies cannot be performed until the transmitters used by these inputs are known.

One of the most critical tests of a transmitter substance is the demonstration of its release from specific fibres. Accordingly, as a first approach to the identification of the transmitters used by hippocampal afferents, we analysed the efflux from hippocampal slices of the excitatory amino acids, aspartate and glutamate. Aspartate and glutamate depolarise neurones in the central nervous system⁶, and they are present in large amounts⁷. They are thus putative excitatory transmitters, although they have not been clearly related to specific fibre projections. We now report that aspartate and glutamate are released from superfused slices of hippocampal regions in a Ca^{2+} -dependent manner and that their efflux is associated in part with two excitatory afferents, the hippocampal commissural fibres and the perforant path.

In preliminary experiments, slices of rat dentate gyrus and regio superior were depolarised with 56 mM K^+ in Ca^{2+} -free medium into which Ca^{2+} was then introduced (Fig. 1). The rate of efflux of glutamate and aspartate was elevated somewhat by depolarisation of the tissue in the absence of Ca^{2+} , but the subsequent introduction of Ca^{2+} into the medium increased the efflux rate more dramatically (by 500–1,100 pmol per 30 s for a standard number of slices). This effect of Ca^{2+} was nearly always evident within 30 s. In other studies, the Ca^{2+} -evoked efflux was shown to be dependent on depolarisation of the tissue (with K^+ or veratridine); addition of Ca^{2+} to medium containing only 6 mM K^+ did not increase the rate of efflux.

Since physiological transmitter release invariably requires Ca^{2+} (ref. 8), it seemed quite possible that the Ca^{2+} -dependent portion of the depolarisation-evoked efflux was derived from synaptic boutons. In view of this relationship between Ca^{2+} and transmitter release, we tested whether boutons of the commissural or entorhinal projections to the hippocampal formation could have been responsible for the Ca^{2+} -dependent efflux of aspartate or glutamate. The commissural fibres arise from hippocampus regio inferior and terminate in all three regions of the contralateral hippocampal formation—dentate gyrus, regio inferior and regio superior^{9,10}. The perforant path fibres originate in the entorhinal cortex and make synaptic contact with the granule cells of the dentate gyrus^{11,12}. Some fibres of entorhinal origin also terminate in regio inferior and regio superior. In these experiments, either the contralateral hippocampal formation or both entorhinal cortices were removed, and slices of dentate gyrus and regio superior were cut, incubated and superfused as before 8–9 d after operation. We reasoned that, if aspartate or glutamate were the transmitter of the commissural or entorhinal projections, its Ca^{2+} -dependent

efflux should have been reduced by eliminating the appropriate fibre tract.

By comparison with paired, sham-operated control groups, the Ca^{2+} -dependent efflux of glutamate from the dentate gyrus, but not from regio superior, was reduced almost 50% by a bilateral entorhinal lesion (Table 1). Efflux in the absence of Ca^{2+} was unaffected. Our data indicate that about half the Ca^{2+} -dependent efflux of glutamate from slices of the rat dentate gyrus is associated with the perforant path fibres, and glutamate may therefore be their transmitter.

Boutons which utilise aspartate and glutamate as transmitters are thought to possess a high affinity uptake mechanism for these amino acids^{13,14}. In accordance with the postulated role for glutamate as a transmitter of the perforant path, a bilateral entorhinal lesion reduced the uptake of labelled glutamate (at a concentration below the K_m of the high affinity uptake system¹³) into slices of the dentate gyrus, but not into slices of regio superior (Table 2). In the same experiments, the high affinity uptake of GABA was unaffected by the lesion.

Commissurotomy significantly reduced the Ca^{2+} -dependent efflux of aspartate from both the dentate gyrus and regio superior (Table 1), two regions to which commissural fibres project, but did not affect efflux in the absence of Ca^{2+} . Therefore aspartate is probably released by these fibres and may be the commissural transmitter.

Commissural fibres have been shown to form additional synapses in the dentate gyrus after unilateral¹⁵ or bilateral (unpublished) entorhinal lesions, and these connections begin to assume electrophysiological function 9 d after operation¹⁶. One would expect some increase in the Ca^{2+} -dependent efflux of the commissural transmitter at this time. In agreement with the hypothesis that aspartate is released from boutons of the

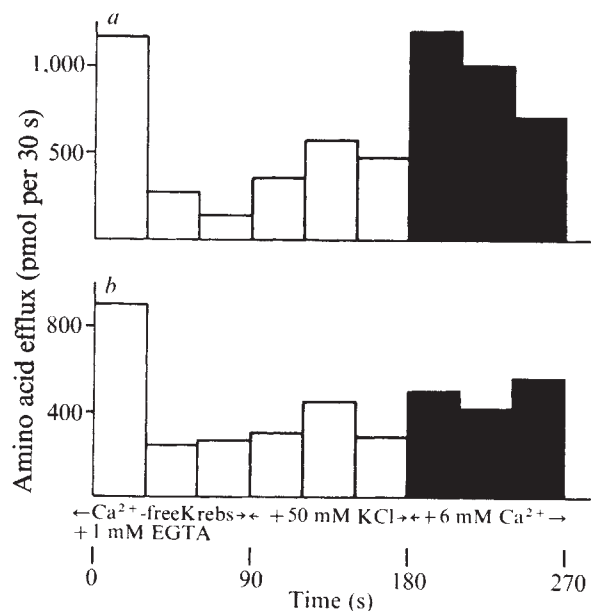


Fig. 1 Time course of glutamate (a) and aspartate (b) efflux from slices of the rat dentate gyrus. Slices of about 300 μm thickness cut from 12 1-mm thick coronal slabs¹⁸ of dentate gyrus (about 11 mg wet weight) were used in each experiment. After a 10-min incubation in Ca^{2+} -free Krebs bicarbonate medium containing 1 mM EGTA and 10 mM glucose, the slices were loaded by gentle suction on to a glass fibre filter, which was then inserted into a continuous superfusion device¹⁹. The slices were initially superfused at 10.8 ml min^{-1} for 90 s with Ca^{2+} -free, glucose-free, Krebs bicarbonate medium containing 1 mM EGTA. Then the K^+ concentration was increased and finally Ca^{2+} was introduced into the medium. The superfusate fractions were desalted with cation-exchange columns, and the glutamate and aspartate contents were determined by micro-enzymatic fluorometric methods²⁰. The bars represent mean values for 13 (glutamate) or 7 (aspartate) experiments. Similar results were obtained with slices of regio superior.

Table 1 Ca^{2+} -stimulated efflux after lesions of hippocampal afferents

	Control (maximal increase over baseline in pmol per 30 s*)	Lesion (% of control)
Commissurotomy		
Aspartate		
Dentate gyrus	690 $\pm$ 180	44 $\pm$ 19(6)†
Regio superior	940 $\pm$ 270	56 $\pm$ 11(5)‡
Glutamate		
Dentate gyrus	710 $\pm$ 210	91 $\pm$ 37(5)
Regio superior	820 $\pm$ 150	64 $\pm$ 9(5)‡
Bilateral entorhinal lesion		
Aspartate		
Dentate gyrus	810 $\pm$ 260	153 $\pm$ 13(7)§
Regio superior	750 $\pm$ 170	192 $\pm$ 42(5)
Glutamate		
Dentate gyrus	1080 $\pm$ 180	54 $\pm$ 11(7)§
Regio superior	1130 $\pm$ 360	85 $\pm$ 19(4)

Slices were cut from 12 1-mm thick coronal slabs of hippocampal formation and superfused (see Fig. 1). Slices from operated and control animals were used in consecutive experiments conducted in random order on the same day. Controls received sham lesions. Values are expressed as means $\pm$ s.e.m. for the number of experiments in parentheses.

*The amount of Ca^{2+} -dependent efflux was calculated by subtracting the efflux rate immediately preceding the Ca^{2+} stimulus (previously shown to remain approximately constant, if Ca^{2+} was not introduced) from the peak efflux rate during stimulation with Ca^{2+} .

Results differed from control at † $P < 0.05$, ‡ $P < 0.02$, § $P < 0.01$ (two-tailed, paired t test). For all other differences $P > 0.05$.

commissural fibres, the Ca^{2+} -dependent efflux of aspartate from slices of the dentate gyrus was increased 8–9 d after a bilateral entorhinal lesion.

In 4 of 5 experiments, the Ca^{2+} -dependent efflux of aspartate from slices of regio superior was also increased by a bilateral entorhinal lesion. We do not know, however, whether the commissural fibres form additional synapses in regio superior after this lesion.

The Ca^{2+} -dependent efflux of glutamate from slices of regio superior, but not from dentate gyrus slices, was also reduced by a commissurotomy. This effect was not attributable to inadvertent section of the crossed temporo-ammonic tract, fibres of entorhinal origin which innervate the contralateral hippocampus^{9,17}, since a bilateral entorhinal lesion would then have produced a similar result. Possibly some of the commissural fibres which innervate regio superior release glutamate or else glutamate is released by some other crossed pathway that was damaged.

In summary, the results presented here demonstrate lesion-induced changes in Ca^{2+} -dependent excitatory amino acid efflux from slices of hippocampal regions which are selective

Table 2 Uptake of glutamate and GABA into slices after bilateral entorhinal lesion

		^3H -GABA (d.p.m. per 10 min $\times 10^{-6}$)	^{14}C -Glutamate (d.p.m. per 10 min $\times 10^{-5}$)
Dentate gyrus	Sham	5.15 $\pm$ 0.21	3.77 $\pm$ 0.30
	Lesion	5.20 $\pm$ 0.42	2.96 $\pm$ 0.25*
Regio superior	Sham	4.20 $\pm$ 0.60	5.05 $\pm$ 0.98
	Lesion	4.38 $\pm$ 0.36 (5)	4.68 $\pm$ 0.37 (6)

Immediately before the preliminary 10-min incubation of slices from rats with bilateral entorhinal lesions (see Fig. 1), 2,3- ^3H -GABA (10 μCi , 36.7 Ci mmol^{-1}) and U- ^{14}C -glutamate (1 μCi , 237 mCi mmol^{-1}) were added at a final concentration of 0.03 μM and 0.4 μM respectively. The amount of radioactivity taken up and retained by the tissue was calculated by adding the radioactivity released during superfusion to that still present in the tissue after superfusion was terminated. Values are expressed as means $\pm$ s.e.m. for the number of experiments in parentheses.

*Slices from animals with bilateral entorhinal lesions differed from control at $P < 0.05$ (two-tailed, paired t test).

for the type of lesion, the hippocampal region and the particular amino acid. On this basis, we suggest aspartate as a strong candidate for transmitter of the hippocampal commissural fibres and glutamate for transmitter of the perforant path fibres. The possibility has not, however, been excluded that the lesions induce specific reductions in amino acid efflux from undamaged cellular elements or that substances other than those tested are also released by the fibre tracts in question. In any case, these studies should aid our understanding of adaptive mechanisms within hippocampal afferents.

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γ -Aminobutyric acid in rat superior cervical ganglion

RADIOLABELLED γ -aminobutyric acid (GABA) is taken up by both neurones and glial cells in the central nervous system^{1,2}, where it mixes with endogenous GABA stores³. Superior cervical and dorsal root ganglia also accumulate radioactive GABA and this amino acid is taken up exclusively in satellite glial cells^{4,5}. The presence of GABA in peripheral neural tissues has not, however, been demonstrated unequivocally⁶⁻⁸. We have measured GABA and glutamic acid in rat superior cervical and nodose ganglia by mass fragmentography. The concentration of GABA in nodose ganglia was lower than in the superior cervical sympathetic ganglion (SCG). Moreover, our results suggest that the content of GABA in the SCG may be controlled by neuronal activity.

Sprague-Dawley rats (Zivic Miller) were killed by exposure of their heads and necks to a focused microwave beam for 2.2 s (ref. 9) before the dissection of SCG and nodose ganglia. In other experiments, where the effect of preganglionic stimulation on the GABA content of SCG was studied, the rats were not killed by microwave (see below). Glutamic acid and GABA were determined according to the method of Bertilsson and Costa¹⁰. Briefly, tissues were homogenised in 150 μ l of 80% aqueous ethanol containing 330 pmol of δ -aminovaleric acid and 7.5 nmol of pentadeuterium-labelled glutamic acid used as internal standards to quantitate GABA and glutamic acid, respectively. After centrifugation, the supernatant was

evaporated to dryness and the residue was reacted with 50 μ l of 1,1,1,3,3,3-hexafluoroisopropanol and 100 μ l of pentafluoropropionic anhydride at 60 °C for 1 h, in a small sealed vial. The pentafluoropropionyl amide hexafluoroisopropyl ester of GABA, glutamic acid and their internal standards are formed during this reaction. The reagent was evaporated and the residue was dissolved in 10 μ l of ethyl acetate. About 3 μ l of the solution was injected into a gas chromatograph-mass spectrometer (LKB model 9000 with a multiple ion detector). The temperature of the column (1.5 m $\times$ 3 mm internal diameter packed with 3% OV-17 on Gas Chrom Q) was 112 °C. The ionising potential was 80 eV and the trap current was 60 μ A. To ascertain absolute specificity of the analysis, the intensity of three characteristic fragments of GABA (*m/e* 176, 190, and 204) and two of glutamic acid (*m/e* 202 and 230) were monitored in each determination. The mean of the relative intensity of these fragments from the SCG deviated less than 2% from those of standards. The fragments *m/e* 204 and 206 were used to monitor the internal standards of GABA and glutamic acid, respectively.

The GABA content of SCG was 297 ± 18 pmol per mg protein (Table 1). Although this concentration is only a few per cent of that in brain, it is of the same order of magnitude as dopamine and noradrenaline content of SCG¹¹. The GABA concentration of nodose ganglion, which has only a few synapses, was about 106 pmol per mg protein. At this low concentration, no absolute identification (by the three fragments *m/e* 176, 190, and 204) could be obtained; however, it can be stated that the GABA concentration in SCG is at least 3 times greater than in the nodose ganglion. The concentration of glutamic acid in the SCG (Table 1) agrees with that reported by Nagata *et al.*⁶.

To determine if GABA stored in SCG is in a dynamic equilibrium the GABA content in SCG was measured 4 h after amino-oxyacetic acid injections (50 mg kg⁻¹ of the hemihydrochloride intraperitoneally). Amino-oxyacetic acid, an inhibitor of GABA transaminase, the enzyme responsible for the catabolism of GABA¹², increased the GABA content of SCG more than fourfold (Table 1). Chlorisondamine HCl (10 mg kg⁻¹ intraperitoneally) given 8 h before killing, SCG decentralisation (7 d before) and urethane (1,500 mg kg⁻¹ intraperitoneally, 20 min before) decreased the GABA content of SCG by about 50% (Table 1). Amino-oxyacetic acid, decentralisation or urethane did not change the glutamic acid level in SCG. Thus, the GABA level was decreased by (1) blocking the ganglionic nicotine receptors, (2) destroying the preganglionic nerve terminals and (3) giving an anaesthetic agent that may act by decreasing the stimuli originating from the central nervous system and reaching the SCG¹³. These results indicate that GABA in SCG may be under neuronal control. The observation that only 50% of the GABA content disappeared after decentralisation indicates that the remaining GABA may either

Table 1 Concentration of GABA and glutamic acid in rat superior cervical ganglion

Treatment	GABA pmol per mg protein	Glutamic acid nmol per mg protein
Saline	297 $\pm$ 18 (16)	27.5 $\pm$ 2.6 (4)
Amino-oxyacetic acid (50 mg kg ⁻¹ ; intraperitoneally; 4 h)	1,348 $\pm$ 147 (4)†	36.0 $\pm$ 3.6 (4)
Chlorisondamine (10 mg kg ⁻¹ ; intraperitoneally; 8 h)	143 $\pm$ 11 (4)†	NA
Decentralised	137 $\pm$ 11 (8)†	31.1 $\pm$ 0.9 (4)
Urethane (1,500 mg kg ⁻¹ ; intraperitoneally; 20 min)	154 $\pm$ 16 (4)*	30.0 $\pm$ 3.7 (4)

Animals were killed by focused microwave beam. Values are mean $\pm$ s.e.m. Number of determinations in parentheses.

* $P < 0.01$; † $P < 0.001$; NA, not assayed.

reflect the low steady state in absence of stimulation or originate from other sources than local synthesis; perhaps, it may be blood borne to the ganglion, where it is taken up by the glial cells.

In other experiments, rats were anaesthetised with urethane ($1,500 \text{ mg kg}^{-1}$ intraperitoneally) and 10–20 min thereafter, both SCG were exposed. One side was stimulated preganglionically at 20 Hz for 60 s (3.5 V, 0.3 ms duration pulse) and the other ganglion was used as a control. Immediately after stimulation, both ganglia (stimulated and unstimulated) were dissected out and immediately frozen on dry ice. The stimulated SCG contained more GABA ($1,486 \pm 73 \text{ pmol per mg protein}$; $P < 0.01$ by paired t test) than the unstimulated ganglia ($1,256 \pm 95 \text{ pmol per mg protein}$). The interpretation of this result is difficult, since in the absence of microwave inactivation of enzymes the process of dissection and isolation by itself increases the concentration of GABA by about 8 times (from 154 ± 16 in urethane-treated and microwaved animals to $1,256 \pm 95 \text{ pmol GABA per mg protein}$ obtained in this experiment). Nevertheless, these data lend support to the hypothesis that GABA stores in SCG are under the control of neuronal activity.

We have demonstrated that the GABA content in the rat SCG is higher than in the nodose ganglion. Since the nodose ganglion has only a few interneurons and synapses, it may be surmised that the GABA storage in nodose and SCG resides in glial cells and can be regulated by the synaptic activity of the tissue. The endogenous GABA present in the glial cells of the SCG is stored against a concentration gradient by the high affinity uptake system which has been demonstrated in SCG⁴. As this GABA seems to be under control of neuronal activity, there may be a functional role of GABA in glial cells. Perhaps, it is released by the high potassium concentration which may be formed in the interstitial fluid during intense neuronal activity. The presence of neurones in the SCG that lack a GABA uptake mechanism but can synthesise GABA cannot, however, be excluded.

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Stimulation-dependent release of ³H-adenosine derivatives from central axon terminals to target neurones

ADENOSINE is postulated to function in the brain as an intercellularly active neurohumoral substance. This has been suggested by *in vitro* studies showing that adenosine can be released from brain tissue by electrical stimulation at quantities which are sufficient to increase the formation of cyclic AMP¹. The finding that adenosine derivatives are released from dendrites and particularly axon terminals points to the neurones as a major source for the released nucleotides². Thus, nucleotides may function as 'secondary' or 'additional' neuronal transmitters

mediating long lasting effects of a neurone on the metabolism and function of its target cells. Further evaluation of these hypotheses will require demonstrations that endogenous adenosine or its derivatives are actually released by synaptic connections in the intact brain and that this process is influenced by activation of these connections. We have carried out experiments in which tritiated adenosine was transported from cell bodies into a population of terminals, and in which the amount of radioactive materials taken up by the target cells of these terminals was measured in the presence and absence of synaptic stimulation. Stimulation was found to increase the release of ³H-adenosine derivatives from central axon terminals to their postsynaptic neurones.

These studies were carried out in rat entorhinal cortex–dentate gyrus projection ('perforant path'). This system was selected because its terminals form a compact, discrete layer in the distal dendrites of the dentate gyrus granule cells. As a result of this arrangement it is possible to microdissect samples of the dentate gyrus which are purely postsynaptic to the entorhinal terminals (that is, the cell bodies of the granule cells) and to compare it with a sample which contains both terminals and postsynaptic (that is, dendritic) elements (see Fig. 1). Finally, because of its laminated organisation, the entorhinal dentate gyrus connection produces easily recordable extracellular physiological responses when activated³; this makes it a simple matter to establish optimal location for the electrical stimulation required in the following experiments.

Adult Sprague–Dawley rats (350–400 g body weight) while under Nembutal anaesthesia were stereotactically injected with ³H-adenosine ($25 \mu\text{Ci} = 2.3 \text{ nmol}$ in $0.5 \mu\text{l}$, specific activity: 11 Ci mmol^{-1}) into the dorsal aspect of the entorhinal cortex. The amounts injected were in the physiological range of the normal tissue content of adenine derivatives ($2\text{--}3 \text{ nmol per mg tissue}$). Using recordings of the extracellular field potentials from the granule cell layer as a guide, a bipolar stimulation electrode was localised to a position in the injection site where it optimally activated the rostral dentate gyrus by way of the

Fig. 1 *a*, Region of perforant path (PP) termination (shaded area) in the hippocampus ipsilateral to the injected entorhinal cortex (EC). Positions of recording (R) and stimulating (S) electrodes as indicated. *b*, Areas sampled in the microdissection procedure (dotted area). M, Molecular layer receiving afferents from the perforant path; G, granule cell layer containing only postsynaptic elements.

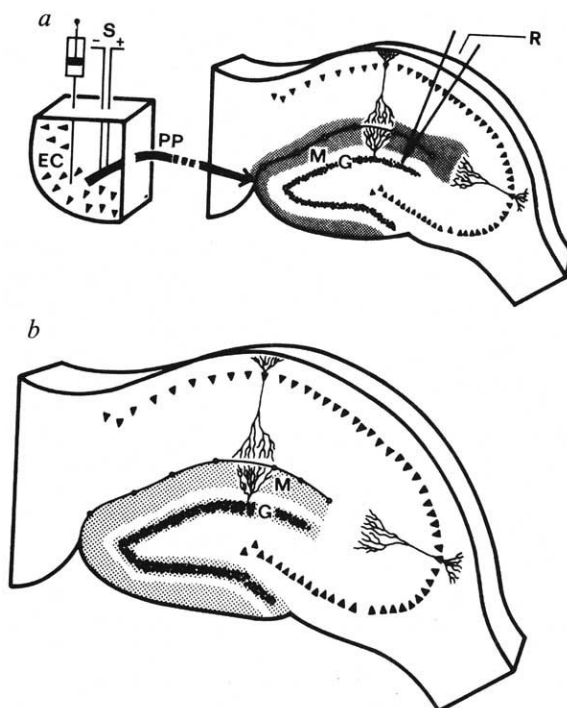


Table 1 Effect of electrical stimulation on release of ^3H -adenosine derivatives from axon terminals to granule cells

Duration of experiments	Ratio $G/(M+G)$		% Increase in stimulated compared with control rats
	Control	Stimulated	
5–8 h	$0.20 \pm 0.03^*$ ($n = 5$)	0.28 ± 0.03 ($n = 2$)	40
18 h	0.19 ± 0.03 ($n = 4$)	0.26 ± 0.01 ($n = 5$)	37
1 d	0.26 ± 0.04 ($n = 3$)	0.36 ($n = 1$)	39
3 d	0.33 ± 0.01 ($n = 3$)	0.36 ($n = 1$)	9

G and M represent measured content of radioactivity (d.p.m.) in granule cell layer (G) and molecular layer (M) samples. ($M+G$) was usually equivalent to 8,000–14,000 d.p.m. at transport times of 18 h and longer. Values are means $\pm$ s.d. n , Number of experiments (rats).

perforant path (see Fig. 1). Both the recording and stimulating electrodes were then cemented in place. The animal was then attached to a cable and placed in a chamber which allowed continuous recording and stimulation while the animal was awake and freely moving. Stimulation of the entorhinal cortex was carried out throughout the experiment. Pulses of 0.10 ms and 4–8 Hz were presented in a cyclic manner (10 min on and 2 min off). Such stimulation was capable of, but did not normally produce, post-tetanic potentiation of the granule cell response at the stimulus intensities used in the experiment. A second group of rats serving as controls were treated and analysed in the same way but received no electrical stimulation. The rats were killed by decapitation at various times after injection and the different samples indicated in Fig. 1 were dissected from 400- μm thick sections of the ipsilateral hippocampal target area. Homologous samples from ten slices were pooled, homogenised, sonified, and their radioactivity measured by scintillation counting. The consistency of the microdissection procedure was checked by measuring the protein content of the samples with the Lowry technique.

In normally behaving, unstimulated rats, the quantities of ^3H -adenosine derivatives released from the entorhinal axon terminals and taken up by the different target neurones in the hippocampus, were found to depend on the density of the entorhinal input to these neurones and the time allowed for transport (our unpublished results). The effect of electrical stimulation is shown in Table 1. For these experiments a period of 18 h was used since, at this time, a high proportion of TCA-soluble ^3H -adenosine derivatives had reached the ipsilateral hippocampus by way of axonal transport. Their concentration at this time was 30 times greater than that of the non-target cortical control tissue. The amount of the transported ^3H -nucleotides channelled to the granule cell target area (including the terminals of the entorhinal axons) is represented by the total radioactivity recovered from the molecular layer (M) and granule cell layer (G) samples. The ratio $G/(M+G)$ gives the relative content of radioactivity in the somata of the granule cells. This value will be an underestimation of the radioactivity in the granule cells since it does not include material in the distal dendrites of these cells. But this ratio represents a useful relative measure for the release to and uptake by the granule cells.

Comparison of stimulated with non-stimulated animals revealed that release and uptake were increased markedly (by about 40%) as a function of electrical stimulation (see Table 1). These results complement the *in vitro* findings of McIlwain¹. They indicate that a functionally dependent release of adenosine derivatives (which is well documented for peripheral monoaminergic⁴, cholinergic^{5,6} and purinergic nerves⁷) also occurs from central neuronal pathways. Since the nucleotide levels in the central nervous system are rather shielded from foreign influences by the blood-brain barrier⁸ they seem to be largely determined by intracerebral recycling processes. A release from

axon terminals may therefore significantly alter the local concentration of adenosine at the target neurones and induce metabolic changes which reflect the amount of input received. It is tempting to speculate whether the unusual physiological plasticity of the entorhinal-dentate gyrus synapses, which seem to be potentiated for hours and perhaps days⁹ after repeated stimulation, is related to adenosine-induced metabolic changes in the granule cells.

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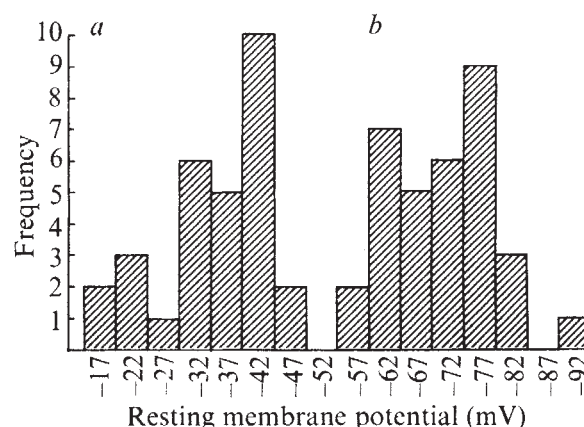
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Membrane potential of juxtaglomerular cells

THE juxtaglomerular granular cell of the kidney is the site of renin storage and release, and is located most commonly within the media of the afferent arteriole, often in close apposition to the distal tubule and to sympathetic nerve endings. Morphologically, it seems to be a smooth muscle cell that has been modified for secretory activity, and contains both myofibrils and membrane-limited renin granules^{1–3}. Evidence has accumulated to suggest that the juxtaglomerular cell, either directly or by way of its neighbouring cells, is stimulated to release renin by catecholamines, by changes in intravascular or interstitial pressures, and by variation in local ionic concentrations⁴. But, even though the juxtaglomerular cell is central to all theories of renin secretion, little effort has been made to investigate its physiology at the cellular level. In this paper, a new method is described for visualising living juxtaglomerular cells, and for recording their electrical activity

Fig. 1 Distribution of resting potentials of juxtaglomerular cells, showing the two groups. *a*, Group I; *b*, group II.

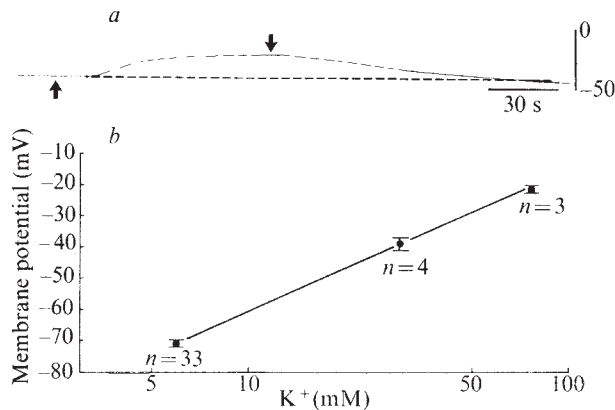


Fig. 2 *a*, Depolarisation by 70 mM K⁺ (first arrow) and repolarisation after return to Ringer's solution (second arrow). Ringer's solution contained (mM): Na⁺ 138.0, K⁺ 5.8, Ca²⁺ 2.5, Mg²⁺ 1.2, Cl⁻ 123.6, SO₄²⁻ 1.2, HCO₃⁻ 25, H₂PO₄⁻ 1.2, glucose 10; and was bubbled with 95% O₂/5% CO₂. Solutions of increasing K⁺ concentration were made by equimolar substitution of KCl for NaCl. *b*, Relationship of membrane potential to log (K⁺)_o.

using intracellular microelectrodes. The results suggest that changes in the membrane potential of the juxtaglomerular cell occur with changes in renin secretion.

At least one stable resting potential was recorded in each of 25 successful experiments. For each experiment both kidneys were removed from a sodium-depleted mouse under ether anaesthesia, sectioned as thinly as possible using a razor blade, and then pressed through a 240-μm sieve. The fragments were stained with neutral red (which stains juxtaglomerular cell granules²), and transferred to a chamber maintained at 37 °C with Ringer's solution flowing through at a rate of 3 ml min⁻¹. Adherence of the fragments to the chamber was achieved by precoating the glass with polylysine (10 mg ml⁻¹, Sigma), a polycationic molecule that fixes tissue to glass electrostatically⁵. Glass microelectrodes, pulled with a fibre inside to tips < 1 μm, were filled by injection with 2 M KCl; tip potentials were < 5 mV, resistances 80–160 MΩ. Using Normarski optics and a ×40 water immersion lens, the juxtaglomerular cells, with their granules stained red, were readily identifiable as chains or clusters clearly distinct from the larger, diffusely stained tubular cells. Impalement by the microelectrodes proved difficult because of the small size and adventitious coats of the juxtaglomerular cells, so that it was only possible to penetrate and record stable potentials from 62 of the more than 1,000 cells in which it was attempted. In general, the potentials were stable for 10–20 s; but in several instances stable potentials were observed for up to 7 min. In those trials in which the membrane potential of a cell remained stable to within 1 mV for at least 7 s, the composition of the surrounding medium was altered by changing either the ionic composition of the flowing Ringer's solution, or by injecting drugs directly into the bath. The juxtaglomerular cells remained viable, as manifested by their exclusion of Trypan blue, for several hours. In contrast, within 1 h the tubular cells generally failed to exclude the dye.

As shown in Fig. 1, the stable resting potentials fell into two distinct populations: a low potential group, with mean -35 mV, and a higher potential group, with mean -70 mV. And, indeed, using a χ^2 test, the data were well approximated using two curves with means -35 ± 8.5 mV (s.d.) and -70 ± 7.9 mV, respectively ($\chi^2 = 15.08$, d.f. = 10, $P > 0.10$); in contrast, the data were poorly fitted by a single curve with mean -54 ± 19.7 mV ($\chi^2 = 37.36$, d.f. = 15, $P < 0.005$). It is possible that the cells with lower potentials represent a less viable group rather than a physiologically distinct population. No obvious morphological differences, however, could be found between the cells,

and the whole range of potentials shown in Fig. 1 could be recorded from different cells prepared at the same time.

Since renin secretion is inhibited by increasing K⁺ concentrations^{6,7}, it was of interest to determine whether K⁺ had a significant role in determining the membrane potential of the juxtaglomerular cells. All cells that were tested ($n = 11$) depolarised with increasing K⁺. Figure 2*a* illustrates the typical response to increased K⁺ concentration, and shows depolarisation with 70 mM K⁺ and repolarisation when normal Ringer's solution was restored in the bath. The potential was related to K⁺ concentration: for cells of group I the relationship was curvilinear, as might be expected from their lower resting potentials⁸, whereas for group II cells the relationship was linear (Fig. 2*b*). The slope, 43 mV per tenfold change in K⁺ concentration was greater than that for many non-excitable cells⁸, but less than if the juxtaglomerular cell were simply a K⁺ electrode—that is

$$E_m = 61 \text{ mV} \log [K^+]_o / [K^+]_i.$$

Adrenaline has been shown to stimulate the secretion of renin *in vivo*⁴ and *in vitro*^{9,10}. As shown in Fig. 3, adrenaline caused a small, but statistically significant ($P < 0.005$) hyperpolarisation for cells over the whole range of resting potentials. Since the typical potential change with any mechanical disturbance was depolarisation, it was unlikely that these changes were artefacts. To determine the possibility of an artefact, injections of Ringer's solution into the bath were carried

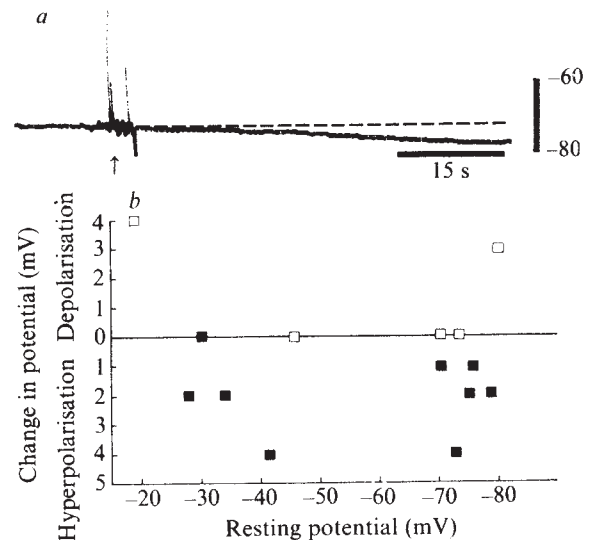


Fig. 3 *a*, Hyperpolarisation of a juxtaglomerular cell by injection (at arrow and artefact) of adrenaline (to an initial bath concentration of 10^{-4} M) into the flowing bath. *b*, Comparison of effects of adrenaline (■) or equal control (□) volume of Ringer's, for cells over the range of resting potentials.

out for six cells. These injections caused either no change or a depolarisation, in sharp contrast to the adrenaline-induced hyperpolarisation ($P < 0.001$). A concentration of 10^{-4} M was used because of its rapid dilution by the flowing bath, and because concentrations of at least this magnitude are needed to stimulate renin secretion *in vitro*^{9,10}.

This investigation provides a fresh approach to the study of the cellular physiology of the juxtaglomerular cell. The trials reported in this paper have demonstrated that two substances (K⁺ and adrenaline) which are known to affect renin secretion in opposite ways, cause the membrane potential to change in opposite directions. These results, although distinctive, do not imply that either adrenaline or K⁺ are the most important controlling elements in renin secretion, or that the membrane potential changes are the cause of subsequent alterations in renin release. Indeed, in other secretory systems, potential changes and secretion may be uncoupled¹¹.

Note, however, that both the adrenaline- and K⁺-induced

potential changes are similar to those that occur in smooth muscle cells¹², which juxtaglomerular cells resemble morphologically. One additional feature of the smooth muscle membrane potential is its sensitivity to stretch¹². In view of the baroreceptor theory of renin secretion¹³, it is tempting to postulate a similar mechanical sensitivity for the juxtaglomerular cell membrane potential. Thus, renin secretion could be partially controlled by changes in arteriolar or interstitial pressures near the juxtaglomerular cell, as signalled by changes in its membrane potential.

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19-Hydroxyprostaglandin E₁ as a major component of the semen of primates

As their name implies, prostaglandins were first identified in secretions of the male reproductive tract. In the early 1930s several workers¹⁻³ showed that human semen could stimulate various smooth muscle preparations, and this activity was ascribed to an unidentified component which von Euler named 'prostaglandin'. von Euler^{4,5} then found that a watery alcoholic extract of rhesus monkey seminal vesicles markedly

lowered the blood pressure in the rabbit but, unlike human seminal vesicle extracts, it had little or no action on the isolated intestine and the uterus of the rat and other laboratory animals. He therefore postulated the existence of another substance in the monkey which he named 'vesiglandin' and which he suggested could be a component of human seminal 'prostaglandin'.

We have shown⁶ that the major prostaglandins present in human semen are two hitherto unidentified compounds, 19-hydroxyprostaglandin E₁ (19-OHPGE₁) and 19-hydroxyprostaglandin E₂ (19-OHPGE₂), and this has since been confirmed⁷. Human semen also contains appreciable amounts of prostaglandins E₁ (PGE₁) and E₂ (ref. 8) which are known to both lower blood pressure and stimulate smooth muscle in test animals. It was therefore interesting to know whether these new 19-OHPGs had any pharmacological activity, and how widely they were distributed throughout the animal kingdom.

Ejaculates were obtained from men, the great apes and various monkeys and domestic and laboratory animals, either by electroejaculation⁹, masturbation, or the use of an artificial vagina, and examined for the presence of 19-OHPGE₁ and 19-OHPGE₂ by gas chromatography-mass spectrometry for identification and gas chromatography alone for quantitation¹⁰. Table 1 shows that large amounts of these two new PGs were recovered from the semen of man, the chimpanzee, orang-utan, rhesus monkey and stump-tailed macaque, and a trace was obtained from a small and possibly incomplete ejaculate from a gorilla, whereas they were undetectable in the semen of domestic and laboratory animals. Figure 1 shows a gas chromatographic trace of the acidic fraction of rhesus monkey semen, in which 19-OHPGE₁ is obviously the main component.

We have already demonstrated that the production of these PGs in men is highly androgen dependent, since the concentrations in the ejaculate fell within 48 h of cessation of testosterone replacement therapy to hypogonadal individuals¹¹. To investigate the site of origin of these compounds, we dissected out the complete male reproductive tract from a freshly killed stump-tailed macaque (*Macaca arctoides*) and analysed each of its components. There was an extremely high concentration of 19-OHPGE₁ and 19-OHPGE₂ in the large seminal vesicles (70 µg g⁻¹; combined seminal vesicle weight 73 g), but none was detectable in the testis, epididymis, vas deferens, ampulla of the vas or prostate. Thus there was sufficient 19-OHPGE₁ and 19-OHPGE₂ stored in the seminal vesicles to account for

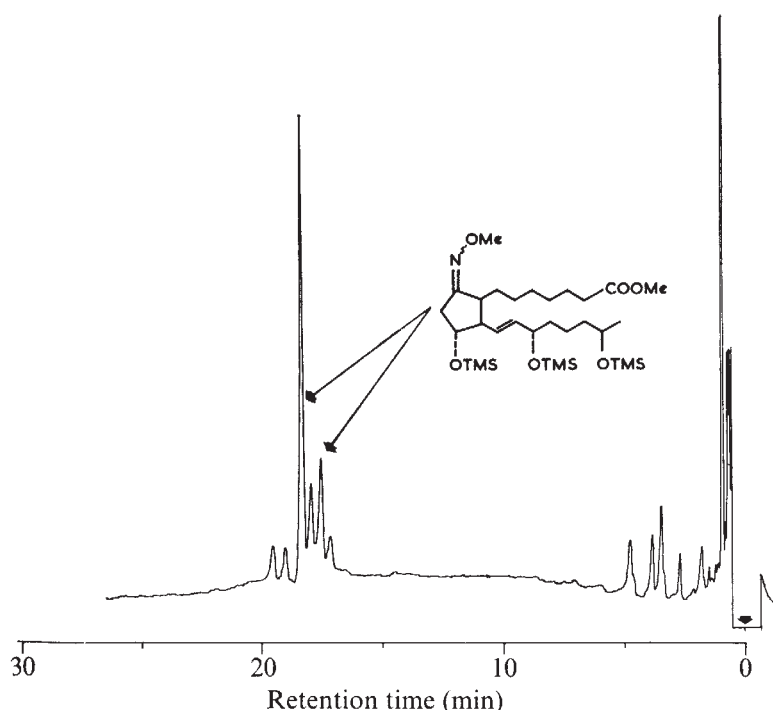


Fig. 1 Gas chromatographic (GC) trace of acidic fraction from semen of *Macaca mulatta*. Coagulated semen (2 g) was stored in methanol at -20°C , homogenised in the same methanol then centrifuged and the methanol evaporated. The residue was treated with 5 ml of a solution of methoxyamine hydrochloride (10 mg ml⁻¹) in pyridinium acetate (1.5 M pH 5). The mixture was left in an ultrasonic bath at 60°C for 40 min then extracted with ether-ethylacetate (3:1); the ethereal layer was evaporated, transferred to a small tube and reacted with diazomethane followed by *bis*-(trimethylsilyl) trifluoro acetamide (20 µl). This solution was injected directly into the GC after 40 min at 60°C . The trace was obtained from a combined gas chromatograph-mass spectrometer (duPont 490B spectrometer coupled to a Varian 1400 GC) the column was a 25-m SCOT column (SGE Ltd) temperature programmed from 170°C to 270°C at 4°C per min. Helium carrier gas flow was 3 ml min⁻¹. Detection was by total ion current monitor.

Table 1 Concentrations of 19-OHPGE₁ and 19-OHPGE₂ in semen

Species	Source of semen	No. of samples	19-OHPGE ₁ concentration mean ($\mu\text{g ml}^{-1}$)	19-OHPGE ₂ concentration mean ($\mu\text{g ml}^{-1}$)
Man (<i>Homo sapiens</i>)	Masturbation	6	100 $\pm$ 47 s.d.	96 $\pm$ 43 s.d.
Chimpanzee (<i>Pan troglodytes</i>)	Masturbation	2	390	84
Gorilla (<i>Gorilla gorilla</i>)	Electroejaculation	1	5	Not identified
Orang-utan (<i>Pongo pygmaeus</i>)	Electroejaculation	2	54	Not identified
Rhesus monkey (<i>Macaca mulatta</i>)	Masturbation	8	420	84
Stump-tailed monkey (<i>Macaca arctoides</i>)	Masturbation	1	780	150
Horse (<i>Equus caballus</i>)	Artificial vagina	1	ND	ND
Cow (<i>Bos taurus</i>)	Artificial vagina	3	ND	ND
Sheep (<i>Ovis aries</i>)	Artificial vagina	8	ND	ND
Pig (<i>Sus scrofa</i>)	Artificial vagina	2	ND	ND
Rabbit (<i>Oryctolagus cuniculus</i>)	Artificial vagina	3	ND	ND
Chicken (<i>Gallus domesticus</i>)	Lumbar massage	1	ND	ND

ND Not detectable, concentration less than 1 $\mu\text{g ml}^{-1}$.

the amount present in a single ejaculate (Table 1). We examined a series of spontaneous ejaculates produced by one stump-tailed macaque that masturbated 11 times in less than 2 h (Table 2). Since the concentration of 19-OHPGEs did not decrease with time, this suggests that the vesicular store can be replenished rapidly. The high concentration of 19-OHPGE₁ in the semen of the stump-tailed macaque has also enabled us to isolate the compound as pure crystals (melting point 129–131 °C) by selective extraction with organic solvents, followed by Sephadex LH20 chromatography.

Dr N. S. Crossley of ICI Pharmaceuticals has synthesised 19-OHPGE₁ and 19-OHPGE₂ for us; however, these two synthetic compounds are each a mixture of the two racemic forms, [(+) and (–)], and the α and β epimers at C₁₉, whereas the naturally occurring 19-OHPGE prostaglandins are probably the (–)-racemate and the 19 β OH epimer¹². Preliminary evidence shows that synthetic 19-OHPGE₁ and 19-OHPGE₂ are about a quarter as active as PGE₁ and PGE₂ in relaxing spontaneously contracting non-pregnant human uterine muscle strips *in vitro*, whereas in contrast to PGE₁ and PGE₂, they have little or no action on rat uterine muscle *in vitro* (unpublished results of J. A. Russell, P. L. T. and R. W. K.). Since only a quarter of the synthetic material corresponds to the naturally occurring compound, it seems that the 19-OHPGEs are comparable in activity with the PGEs

as far as the human uterus is concerned; moreover they are usually present in larger amounts.

In contrast to human semen, with its mixture of 19-OHPGEs and PGEs, the semen from the two species of macaque contains approximately 100 times more 19-OHPGEs than PGEs. This could go some way towards explaining the different spectrum of pharmacological activity that von Euler found between human and rhesus monkey seminal vesicles.

Our study certainly emphasises the widespread occurrence of these new 19-OHPGEs throughout the primates, and the high concentrations that are present. It remains to be seen whether their potent smooth muscle relaxing activity is a significant factor in sperm transport through the human female reproductive tract.

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Table 2 19-OHPGE concentrations in repeated ejaculates from a masturbating stump-tailed macaque (*M. arctoides*)

No.	Time	Weight of ejaculate collected* (g)	Combined concentration of 19-OHPGE ₁ and 19-OHPGE ₂ ($\mu\text{g g}^{-1}$)
1	1100	1.71	461
2	NR	2.17	473
3	NR	0.93	439
4	NR	0.95	189
5	NR	1.07	426
6	NR	0.80	485
7	1150	0.71	496
8	NR	0.79	383
9	1205	0.73	444
10	1215	0.94	341
11	1250	0.97	433
			Total 4.96 mg

* This is a minimal estimate of the total ejaculate produced since the animal invariably ate some of it before it could be removed from the floor of the cage.

NR, Not recorded.

Insulin stimulates active sodium transport in toad bladder by two mechanisms

INSULIN has been shown to increase sodium and potassium fluxes in a series of tissues including muscle¹, and colon² and the isolated perfused kidney³. Attempts to define the exact mechanisms by which insulin stimulates ion transport in these tissues have been hindered by their anatomical complexity and heterogeneous cell populations. Tracer flux studies, using ²²Na, in frog muscle¹ and toad bladder⁴ have suggested a direct stimulatory action of insulin on the sodium pump. Preincubation of intact frog muscle with insulin for 1 h has been shown to increase the amount of ouabain binding by this tissue suggesting that insulin unmasks latent sodium pumps in this structure⁵. The urinary bladder of the toad (*Bufo marinus*) has been shown to transport sodium actively from the urinary (mucosal) side to the blood (serosal) side and has been used extensively to study cation transport and the effects and mode of action of numerous hormones and drugs⁶⁻⁸. We have now examined the mechanisms by which insulin increases short circuit current (a measure of active sodium transport) in the toad bladder.

To define the effects of insulin on short circuit current (SCC), paired hemibladders were mounted in standard amphibian Ringer's in Ussing lucite chambers and the maximum SCC in response to increasing concentrations of the hormone was determined. Figure 1 shows the results of these experiments. The hormone was added to the serosal bathing media since insulin was without effect when added to the mucosal media. Concentrations of insulin greater than 50 mU ml⁻¹ resulted in progressive increases in SCC. The peak response in SCC after addition of 100 mU ml⁻¹ of insulin occurred at approximately 30 min and SCC returned to control values within an hour. Higher concentrations of insulin (250 and 500 mU ml⁻¹) resulted in persistently elevated SCC. An early increase in SCC was seen in the first 5 min after addition of insulin. This increase was similar but not identical to the rapid rise observed after vasopressin, a rise in Na transport which has been shown to be mediated by an increase in the cellular levels of cyclic AMP⁹. The prolonged stimulatory effect of higher concentrations of insulin on SCC was analogous to the response observed after addition of aldosterone, a phenomenon which has been shown to be dependent on protein synthesis^{8,10}.

We next explored the effects of addition of vasopressin and insulin alone and in combination on SCC. These results are shown in Fig. 2. Addition of vasopressin alone (100 mU ml⁻¹)

Fig. 1 Effects of 100 (○), 250 (△) and 500 mU ml⁻¹ (□) of insulin on short circuit current (SCC). Values are mean ± s.e. of 10 experiments.

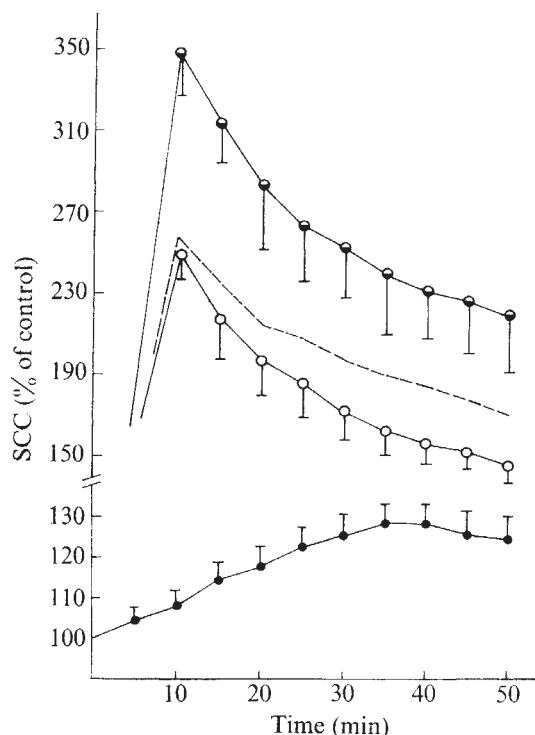
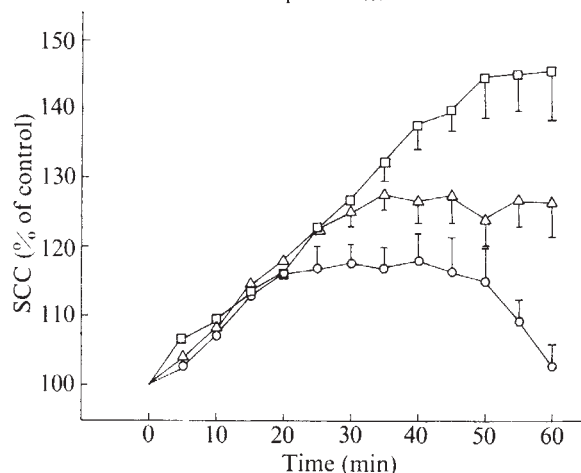


Fig. 2 Addition of 100 mU ml⁻¹ of vasopressin alone (○), 250 mU ml⁻¹ of insulin alone (●) or vasopressin and insulin (—●—) on short circuit current. — — —, Arithmetic sum of the effects of vasopressin and insulin when added alone. Values are the mean ± s.e. of 10 experiments.

resulted in a characteristic rapid increase in SCC which peaked at approximately 250% of control values. Addition of insulin alone increased SCC by approximately 30% about 35 min after addition of the hormone. When insulin and vasopressin were added simultaneously, at the same concentrations as when added alone, a marked enhanced synergistic response in SCC was observed with values rising to 340% of control. The simultaneous addition of insulin plus vasopressin thus produced an increase in SCC far exceeding the arithmetic sum of the responses observed when each hormone was added alone. In other experiments, a similar enhanced response in SCC was seen in bladders treated with insulin at time zero when vasopressin was added 40 min later. This synergistic effect was not observed, however, when the order of addition was reversed and vasopressin was added before insulin. These data suggest that insulin is capable of unmasking latent or reserve sodium pump sites not normally available to stimulation by vasopressin. An allosteric effect of membrane-bound insulin on active sodium pump sites could account for this phenomenon. An alternative explanation would require insulin to enhance or facilitate the formation of cyclic AMP in response to vasopressin, thereby acting as a modulator of vasopressin activity. To explore this possibility further, additional experiments were conducted and the results are shown in Table 1. The cyclic AMP content of scraped toad bladder cells incubated with diluent (control), insulin 250 mU ml⁻¹, vasopressin 100 mU ml⁻¹ and insulin plus vasopressin were studied. Incubation time was 30 min for the experiments reported in column I. In column II the cells were preincubated with insulin for 1.5 h before adding vasopressin for the final 30 min. In neither case did insulin stimulate baseline cyclic AMP concentration nor did it increase cyclic AMP production in response to vasopressin. These data suggest that neither the early nor late stimulatory effect of insulin on SCC is mediated by cyclic AMP and that the enhanced response in SCC seen with the combination of insulin and vasopressin is not dependent on increased production of cyclic AMP. Addition of 0.5 mM cyclic AMP and insulin also resulted in a synergistic

Table 1 Cyclic AMP levels in isolated toad bladder cells

	I (pmol per mg protein)	II
Control	34.3 ± 3.0	22.2 ± 3.4
Insulin (250 mU ml ⁻¹)	35.5 ± 5.1	23.9 ± 4.4
Vasopressin (100 mU ml ⁻¹)	72.3 ± 7.2	46.8 ± 4.7
Insulin and vasopressin	76.1 ± 9.1	51.5 ± 6.1

Values are mean ± s.e. of 8 experiments.

enhanced response in SCC which was greater than the arithmetic sum of the increase in SCC observed when insulin and cyclic AMP were added alone. These data support the hypothesis that insulin may act by unmasking latent pump sites or by increasing the sensitivity of existing pump sites to cyclic AMP. Insulin does not seem to modify the binding of vasopressin to receptor sites.

To explore the role of protein synthesis *de novo* in the stimulation of SCC produced by insulin, bladders were preincubated for 1 h in Ringer containing 20 µg ml⁻¹ of actinomycin D, a potent inhibitor of messenger RNA (mRNA) synthesis. Figure 3 shows the changes in SCC observed after addition of insulin to bladders incubated for the preceding 60 min with or without actinomycin D. An identical increase in SCC was observed following addition of insulin in both control and actinomycin D-treated bladders in the first 30 min. After 40 min, however, the SCC of actinomycin D-treated bladders declined and reached baseline values by 70 min. The bladders not exposed to actinomycin D showed persistent stimulation of SCC for several hours after addition of insulin.

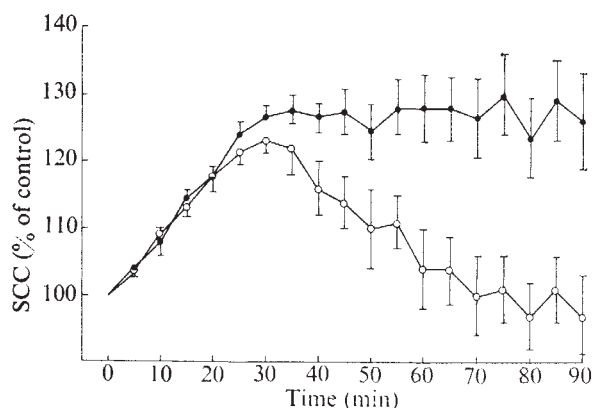


Fig. 3 Effects of addition of insulin at time 0 on the SCC of bladders incubated with (○) and without (●) actinomycin D for the preceding 60 min. Values are the mean ± s.e. of 10 experiments.

The SCC of bladders incubated with actinomycin D alone was no different from that of control bladders incubated without the drug. These data suggest that insulin stimulates active sodium transport (short circuit current) across the toad bladder by two different mechanisms. The rapid initial increase in SCC produced by insulin is unaffected by actinomycin D addition and thus does not seem to depend on protein synthesis. The rapidity of onset also speaks against the involvement of protein synthesis in this phenomenon. This initial phase of increase in SCC produced by insulin is synergistic with the effect of vasopressin, if vasopressin is added after insulin, and suggests that the initial rise in sodium transport produced by insulin may be due to unmasking of latent pump sites within the membrane. The prolonged stimulation of SCC by insulin is inhibited by preincubation of the bladders with actinomycin D. The time course of its appearance and the fact that the persistent elevation of SCC is abolished by an inhibitor of mRNA synthesis suggest that protein synthesis *de novo* is involved in this phenomenon.

It thus seems that insulin stimulates sodium transport in the

toad bladder by two different mechanisms: (1) a rapid phase presumably representing "unmasking" of latent sodium pump sites within the membrane, and (2) a prolonged phase of increased sodium transport that requires synthesis *de novo* of protein. The nature and mode of action of this newly synthesised protein(s) on sodium transport are unknown and further studies will be required to elucidate its role.

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Diminution of tumorigenesis initiated by 4-nitroquinoline-1-oxide by post-treatment with caffeine in mice

THE potent carcinogen 4-nitroquinoline-1-oxide (4NQO)¹ mimics ultraviolet effects^{2,3}. Kondo⁴ proposed an idea to test the mutation theory of cancer by comparison of 4NQO carcinogenesis in mice and 4NQO³ or ultraviolet⁵ mutagenesis in *Escherichia coli*. In parallel with 4NQO mutagenesis in *E. coli*^{2,3,6}, it was concluded that transformation initiated by 4NQO in cultured mouse cells is suppressed by holding in a non-DNA-replicating state⁷, due to elimination of 4NQO-purine adducts⁸ from DNA by an error-free excision repair⁹. There is another type of repair which works after DNA replication and is assumed to be error prone⁵. Again, in parallel with ultraviolet mutagenesis in *E. coli*¹⁰, Kakunaga⁷ has found that transformation of mouse cells by 4NQO is reduced greatly by post-treatment with caffeine. Zajdela and Latarjet¹¹ reported that ultraviolet-inducible skin cancer in mice is suppressed by the presence of caffeine. The present paper reports that post-treatment with caffeine diminishes 4NQO-initiated lung tumorigenesis in mice.

Young (21-d-old) ICR/Jcl mice (9–11 g), 339 males and 285 females were used (Japan Central Laboratory for Experimental Animals). 4NQO (provided by the late Dr K. Mori, Showa Medical School, Tokyo) was dissolved in propylene glycol and caffeine (Nakarai Chemical Co.) in water. Both chemicals were injected subcutaneously; 4NQO in the right flank of animals with a single dose (12.5 µg per g body weight) and caffeine in the left flank with five doses (100 µg per g body weight for each) at intervals of 6–12 h (Fig. 1). These doses of 4NQO and caffeine are the maximum tolerated doses to young ICR/Jcl mice. As described in Fig. 1, animals were divided into five groups. Groups 1 and 2 received caffeine during the 0–36 h and the 120–156 h after 4NQO treatment, respectively, but group 3 received caffeine during the 12–48 h before 4NQO treatment. Group 4 received an equal volume of water, instead of caffeine solution, during the 0–36 h after 4NQO treatment. Solvent control, group 5, received an equal volume of propylene

Table 1 Effects of caffeine on 4NQO-induced tumour yields in mice

No.	4NQO ($\mu\text{g g}^{-1}$)	Caffeine ($\mu\text{g g}^{-1}$)	Period (h) of caffeine treatment after 4NQO	Sex	No. of mice at start	Lung tumours			
						Incidence (%)	χ^2 test*	Tumours per lung (mean $\pm$ s.e.)	<i>t</i> test*
1	12.5	100	0-36	M	100	26/57 (45.6)	NS	1.04 $\pm$ 0.22	NS
				F	85	24/54 (44.4)	$P < 0.05$	1.85 $\pm$ 0.43	$P < 0.05$
2	12.5	100	120-156	M	46	8/26 (30.8)	NS	0.58 $\pm$ 0.20	$P < 0.01$
				F	46	15/28 (53.6)	NS	1.29 $\pm$ 0.38	$P < 0.01$
3	12.5	100	-48--12	M	30	10/22 (45.5)	NS	1.82 $\pm$ 0.69	NS
				F	30	17/28 (60.7)	NS	3.07 $\pm$ 1.11	NS
4	12.5	0.0	—	M	122	41/98 (41.8)	—	1.59 $\pm$ 0.28	—
				F	79	37/57 (64.9)	—	4.42 $\pm$ 1.05	—
5	0.0	100	—	M	41	1/34 (3.0)†	—	0.03 $\pm$ 0.03†	—
				F	45	3/45 (6.7)†	—	0.07 $\pm$ 0.04†	—

* χ^2 test and *t* test were applied for groups 1-3 against group 4. *t* test was applied with the Cochran-Cox approximation, if variance ratio was more than *F* value at 1%. NS, Not significant.

†All 4NQO-treated groups (groups 1-4) developed lung tumours with significantly higher incidence and frequency than solvent control (group 5), ($P < 0.01$). Spontaneous tumour incidence and frequency at week 25 were 2/315 (0.6%) and 0.006 ± 0.005 , respectively.

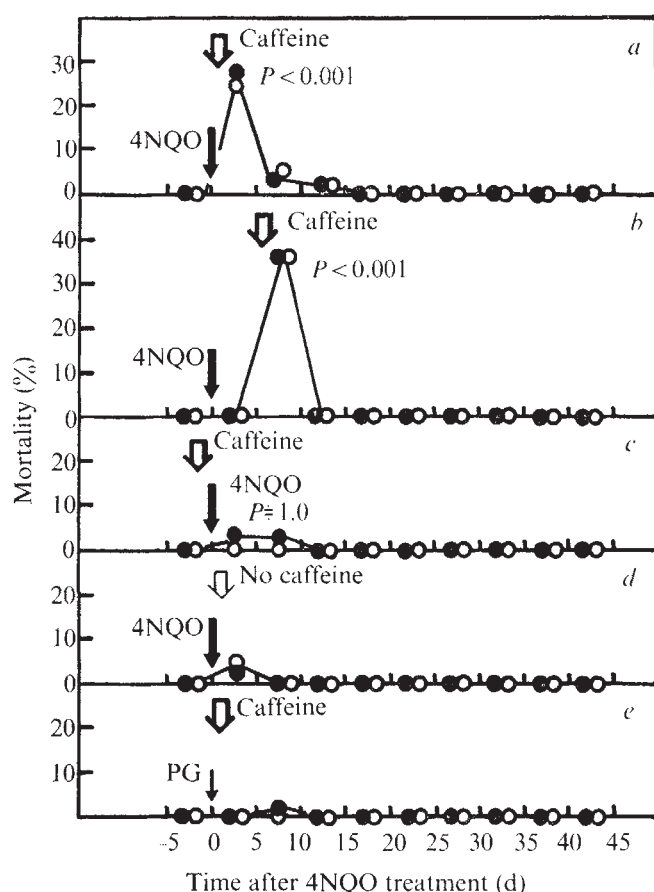


Fig. 1 Enhancement of mortality in 4NQO-treated mice by post-treatment with caffeine. Percentage of dead mice (●, male; ○, female) is plotted against days after 4NQO treatment. Lethality of 4NQO-treated mice confined to 2-5 d after caffeine treatment, primarily due to intestinal death. Numbers of mice used are given in Table 1. χ^2 test was applied with Yates' correction for groups 1-3 against group 4. *a*, Group 1: five subcutaneous injections of caffeine (100 μg per g body weight for each) were given to young mice 0, 6, 12, 24, and 36 h after a single injection of 4NQO at a dose of 12.5 μg per g body weight ($P < 0.001$). *b*, Group 2: five doses of caffeine were given 120, 126, 132, 144, and 156 h after 4NQO treatment ($P < 0.001$). *c*, Group 3: five doses of caffeine were given 12, 24, 36, 42, and 48 h before 4NQO treatment ($P \neq 1.0$). *d*, Group 4: an equal volume of water, instead of caffeine solution, was given after 4NQO on the same time schedule as in *a*. *e*, Group 5: propylene glycol (PG), instead of 4NQO solution, was given, and then followed by five doses of caffeine on the same time schedule as in *a*.

glycol, instead of 4NQO solution, and caffeine during the following 0-36 h. The mice were maintained on mouse diet CA-1 (CLEA)¹², and killed at week 20 after 4NQO treatment to examine lung tumours as described previously^{12,13}. Most lung tumours were papillary adenomas^{12,14}. Lung tumour frequencies, defined as average numbers of tumour nodules per lung, were compared statistically among the experimental groups, since the frequency thus defined increased with doses of carcinogens, even in the high dose range where the increase in the tumour incidence defined as fraction of tumour-bearing mice in survivors levelled off^{12,14}.

Table 1 summarises the results. It will be clear from comparison of groups 1 and 2 with group 4 that in females post-treatment with caffeine during either the early (0-36 h) or late (120-156 h) post-4NQO period suppressed tumour frequency by 60-70%, and that in males caffeine administered during the 120-156 h after 4NQO treatment significantly suppressed the tumour frequency. The tumour frequency in males post-treated with caffeine during the 0-36 h after 4NQO treatment (group 1) was also lower, but not significantly lower, than that of males treated with 4NQO alone (group 4). This will, however, not invalidate the conclusion that caffeine post-treatment suppresses 4NQO-initiated lung tumorigenesis, because the less suppressive effect of caffeine during the 0-36-h period compared with the 120-156-h post-4NQO period was also the case with females (Table 1). Furthermore, it is the rule deduced from 14 sets of our previous experiments with 50-60 young ICR/Jcl mice each, that lung tumour yields are always lower in males than in females after treatment with 4NQO, but not with other carcinogens¹². Caffeine administered during the 12-48 h before 4NQO treatment gave no statistically significant effects on the 4NQO-induced tumour frequencies in both males and females (groups 3 and 4, Table 1). In parallel with the case of cultured mouse cells⁷, post-treatment with caffeine during either the 0-36 h or the 120-156 h after 4NQO treatment, but not pre-treatment, increased mortality in 4NQO-treated mice to about 30% compared with negligible mortality in mice treated with 4NQO alone (Fig. 1). These support the misrepair model⁴ in that caffeine suppresses the repairing action of error-prone post-replication repair on tumorigenic and lethal damage produced in daughter DNA by 4NQO damage on template DNA, resulting in decrease of survivors and induced tumours. The lethal and tumorigenic damage in mice is sensitive to caffeine as late as 120 h after 4NQO treatment (Table 1 and Fig. 1), but cultured cells become insensitive to caffeine as early as 48 h after 4NQO treatment⁷. This difference may simply reflect the fact that a considerable fraction of DNA, bearing 4NQO damage, in the stem cells of the lung remains unreplicated for several days, whereas almost all the cultured cells in the growing state go through the phase of DNA replica-

tion in about one day after 4NQO treatment. Thus I propose that not only 4NQO-induced pretransformational damage in cultured mouse cells but also 4NQO-induced pretransformational damage in mice may be fixed during the first post-4NQO DNA replication period, the fixation being similarly suppressed by caffeine in mice as well as in their cultured mouse cells. It has been reported^{15,16} that actinomycin D inhibits skin tumour formation initiated by 7,12-dimethylbenz(a)anthracene or β propiolactone when given 0 to 14 d later, provided that actinomycin D is present, which probably works as inhibitor for normal DNA synthesis, during the first post-carcinogen DNA replication. Caffeine is known to slow down greatly the post-replication processes and to inhibit specifically post-replication repair in cultured mouse cells after treatment with ultraviolet¹⁷ or 4NQO (M. Morita and M. Tada, personal communication).

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Inhibition of RNA polymerase as a possible anti-leukaemic action of cytosine arabinoside

CYTOSINE arabinoside (ara-C, 1- β -D-arabinosylcytosine) has marked anti-tumour and anti-viral activities^{1,2}, and is currently one of the most successful drugs used to induce remissions in acute granulocytic leukaemia³. Its cytotoxic activity is ascribed to its effect of inhibiting DNA polymerase after being phosphorylated intracellularly to its triphosphate derivative (ara-CTP)⁴. Studies with murine leukaemic cells⁵ and mouse L cell line have indicated⁶, however, that acute cell death induced by ara-C does not completely correlate with the inhibition of DNA synthesis: (1) DNA polymerase activity was rapidly restored after ara-C was removed, whereas cells were still non-viable; (2) the amount of ara-C incorporation into DNA did not correlate with the degree of cell lethality. We have shown previously⁷ that ara-CTP could inhibit RNA polymerase II activity from chicken leukaemic cells when the enzyme was

purified. This report describes results of our kinetic studies characterising the nature of this inhibition.

Chicken leukaemic RNA polymerase IIa, obtained from glycerol-gradient centrifugation as described previously, was used for the present studies. The effect of ara-CTP on the kinetics of incorporation of CTP into RNA was determined

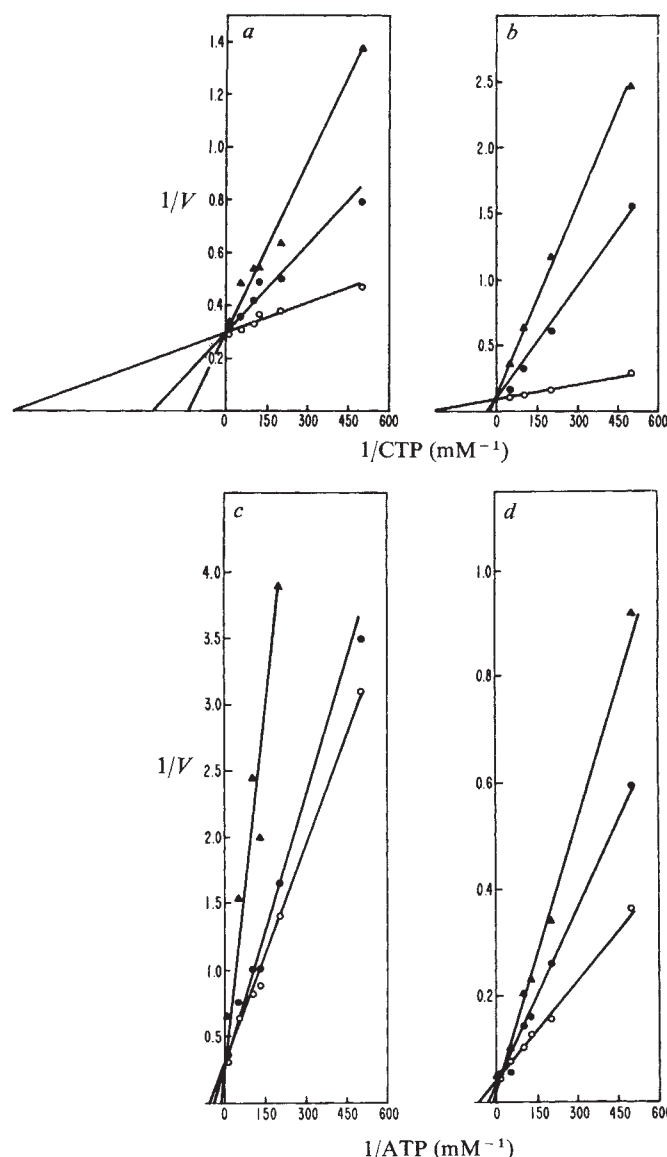


Fig. 1 Effect of ara-CTP and ara-ATP on the kinetics of RNA synthesis (Lineweaver-Burk plot). The reaction mixture (0.1 ml) for chicken leukaemic enzyme (a and c) contained 50 mM (pH 7.9) Tris-HCl, 2 μ g pyruvate kinase, 4 mM phosphoenol pyruvate, 1 mM MnCl_2 , 10 mM KCl, 1 mM dithiothreitol, 40 μ g denatured chicken blood DNA (Calbiochem), 2 μ g RNA polymerase, and four normal ribonucleoside triphosphates, with or without the addition of drug. The reaction mixture for *E. coli* enzyme (b and d) was similar to that described above except that it contained RNA polymerase (b, 0.25 U; d, 0.5 U; fraction IV, Grand Island Biological), 4 mM MgCl_2 , 1 mM MnCl_2 , 5 mM dithiothreitol and 40 μ g native *E. coli* B DNA (Sigma). The four normal ribonucleoside triphosphates used were 0.2 mM GTP, 0.04 mM UTP and ^3H -UTP (1,000 c.p.m. pmol^{-1}); and 0.2 mM ATP and varied CTP concentration (a and b); 0.2 mM CTP and varied ATP concentration (c and d). Incubation was at 37 $^\circ\text{C}$ for 15 min. The reaction was terminated and the RNA precipitated, filtered and counted as described previously⁷. $1/v$ shown on the ordinates is the reciprocal of pmol UMP incorporated into RNA per 0.1-ml reaction mixture per 15 min. Symbols: a (K_i for ara-CTP = 14.9 μM): $\blacktriangle$, 0.1 mM ara-CTP; $\bullet$, 0.03 mM ara-CTP; $\circ$, 0.01 mM ara-CTP; $\circ$, no drug added; b (K_i for ara-CTP = 10.2 μM): $\blacktriangle$, 0.1 mM ara-CTP; $\bullet$, 0.03 mM ara-CTP; $\circ$, 0.01 mM ara-CTP; $\circ$, no drug added; c (K_i for ara-ATP = 150 μM): $\blacktriangle$, 0.1 mM ara-ATP; $\bullet$, 0.03 mM ara-ATP; $\circ$, 0.01 mM ara-ATP; $\circ$, no drug added; d (K_i for ara-ATP = 77.5 μM): $\blacktriangle$, 0.1 mM ara-ATP; $\bullet$, 0.03 mM ara-ATP; $\circ$, 0.01 mM ara-ATP; $\circ$, no drug added.

Table 1 Effects of arabinosyl nucleotides on RNA synthesis by chicken leukaemic RNA polymerase II

Added nucleotide	Control	ara-AMP	ara-ADP	ara-ATP	ara-CMP	ara-CDP	ara-CTP	ara-UTP
UMP incorporated (pmol ml ⁻¹)	27.5	30.5	22.0	1.60	30.4	26.6	1.70	29.4
% control	100	110	80	5.9	110	96	6.3	106

Chicken leukaemic RNA polymerase II (20 µg ml⁻¹) was assayed in the presence of 1 mM arabinosyl nucleotide as indicated. The assay conditions were similar to those described in Fig. 1, except that all four normal ribonucleoside triphosphates used were 0.04 mM, and that the incubation (0.2 ml) was for 45 min. The arabinose nucleotides were obtained from Terra-Marine Bioresearch, La Jolla, California.

and the results are presented in Fig. 1a, using the graphical representation of Lineweaver and Burk⁴. Ara-CTP is a competitive inhibitor for the binding of CTP to RNA polymerase II; K_m (the Michaelis constant) is increased in the presence of the drug without change in V_{max} (the maximum velocity of the reaction). The K_m for CTP and the K_i (enzyme inhibitor dissociation constant) value for ara-CTP inhibition were estimated from 6 determinations to be 1.4 ± 0.5 µM and 14.9 ± 3.0 µM, respectively, for the leukaemic enzyme. A similar study was carried out on *Escherichia coli* RNA polymerase and it was shown that the enzyme was also inhibited by ara-CTP with a K_m (CTP) of 4.2 ± 0.2 µM and a K_i (ara-CTP; from seven determinations) of 10.2 ± 2.7 µM (Fig. 1b).

The reported K_i values for ara-CTP inhibition of DNA polymerase (replication enzyme) were 1 µM for calf thymus⁴, 8.7 ± 5.2 µM for mouse L cells⁸, 18.4 ± 7.9 µM for mouse lymphoma cells⁸. The K_i value reported for Rauscher leukaemia virus reverse transcriptase was 30 µM (ref. 9), and for DNA repair enzyme of isolated hepatocyte nuclei, 500 µM (ref. 10). Our studies with RNA polymerase showed that the K_i value for ara-CTP inhibition for chicken leukaemia enzyme fell in the same range of K_i values for the DNA replication enzyme in mouse L cells and mouse lymphoma cells, and that it was certainly smaller than the values reported for reverse transcriptase and DNA repair enzyme. It indicates comparable affinities of ara-C for the RNA polymerase and the DNA replication enzyme. Because of the competitive nature of this inhibition, the relatively high I_{50} value reported previously⁷, could be explained by the high CTP concentration used in the assay system. It would seem that the degree of inhibition by ara-C of cellular DNA compared with RNA synthesis will depend on the physiological (local) concentrations of dCTP and CTP, the concentration of ara-C used, and their individual K_m values. It has been suggested^{10,11} that inhibition of replicative DNA synthesis by ara-C could not kill cells and that to achieve cell killing ara-C must be given at a dose high enough to impair DNA repair function. At such a high ara-C dosage, however, inhibition of RNA synthesis has undoubtedly occurred.

The anti-tumour and anti-viral activities of adenine arabinoside (ara-A) have long been recognised^{12,13}, although data on clinical trials of this compound are scanty. The compound was suggested to have no effect on RNA polymerase activity^{14,15}, and the inhibition by ara-A of DNA polymerase was shown to be selective¹³. We tested the effects of ara-A on the purified RNA polymerase and found that it inhibited the enzymes by competing with the normal nucleotide ATP. The mean K_m (ATP) and K_i (ara-ATP) values were 17.2 and 150 µM, respectively, for chicken leukaemic enzyme, and were 14.2 and 77.5 µM, respectively, for *E. coli* enzyme (Fig. 1c and d). These K_i values are in the same range as those reported for ara-A inhibition of DNA polymerase¹².

It is well known that ara-C is rapidly deaminated to uracil arabinoside (ara-U) *in vivo*, and that the latter compound has no pharmacological value¹⁶. The negative effect of ara-U on tumour cells prompted us to study the effect of ara-UTP on chicken leukaemic RNA polymerase. The results are presented in Table 1. Ara-UTP did not inhibit the RNA polymerase activity, suggesting that the amino group of cytosine or adenine base may be essential for the inhibition. Further studies showed that the inhibition of RNA polymerase activity by ara-C or ara-A was at the triphosphate level of the drugs. Neither mono- nor diphosphates produced the inhibitory

effect (Table 1). These findings are in accord with *in vivo* observations¹¹ that cellular levels of ara-CTP, but not those of the mono- and diphosphates of ara-C, are the determinants of chemotherapeutic efficacy of ara-C against leukaemia. These, together with the ara-U studies, suggest the possibility of considering RNA polymerase inhibition as the target for ara-C action.

This study has described the inhibitory activity of ara-C (or ara-A) on RNA polymerases. Since RNA polymerase II has been suggested as being responsible for messenger RNA synthesis¹⁷, the inhibition of RNA polymerase II by ara-C may result in the decreased 2-7S RNA synthesis¹⁸ and subsequently in an irreversible cell death, as observed previously^{5,6,18}. Since ara-CTP has apparent affinity for RNA transcription comparable for DNA replication, inhibition of RNA synthesis must be considered before the mechanisms of this drug can be understood completely.

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Similarities in the helical sequences of the repressor-binding sites in the *lac* and λ operators

SEVERAL reports have considered the repressor binding sites in the *lac*¹⁻³ and λ ⁴⁻⁶ operators. The analysis of these regions has focused on features apparent in the linear sequences such as regions of twofold rotational symmetry, and the 17-base-pair sequence which is largely conserved in the rightward and leftward repressor-binding sites of λ ⁶. As these sequences are thought to be wound in the double helical form when they are recognised by repressor^{7,8}, I have investigated whether any further interesting features could be revealed by such an arrangement.

There are many possible ways of "casting" a sequence on a

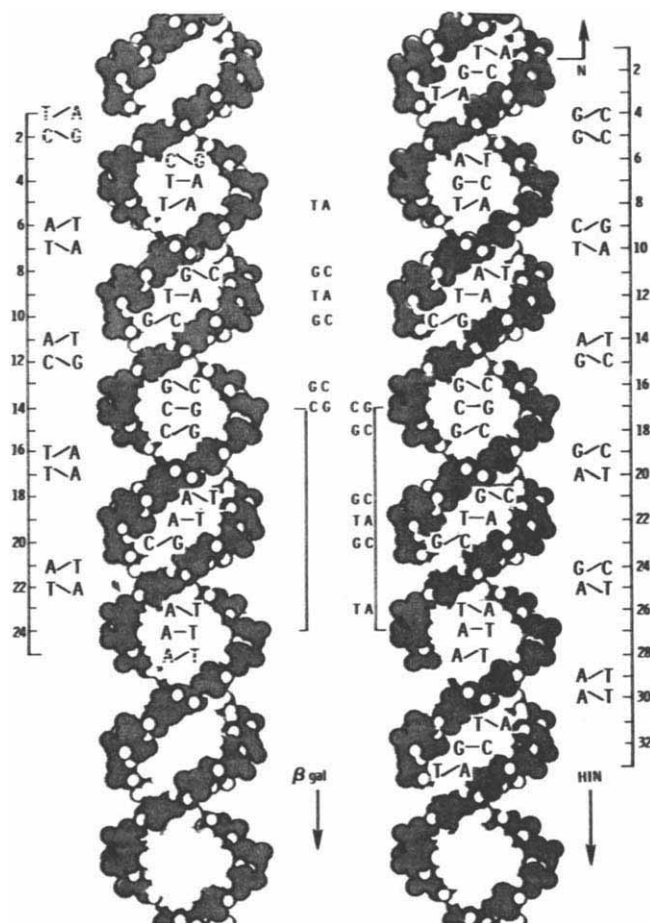


Fig. 1 The *lac* repressor-binding site sequence is shown on the left and the *O_L1* repressor-binding site sequence on the right. The backbone pairs are shown to the side of each helix. The brackets indicate the G-C, A-T couplets. The common face sequences are shown separately in the region between the helices.

DNA helix (formally 10). The effect of starting a sequence on each of the ten different positions in a turn of the helix can be examined using a two-dimensional model of the helix⁹. The sequence is thus seen as it would appear on different "faces" of the helix. I assume here that the visible face of the helix is composed of the three base pairs centred in each groove. The two base pairs aligned most nearly parallel to the line of view at each half turn are assumed to be hidden behind the backbone and therefore not seen on the forward face. Thus a "face sequence" is the sequence presented by the base pairs visible in the grooves with the backbone pairs omitted. Therefore, the sequence one sees on a particular face is composed of the three base pairs in the centre of, say, a wide groove, then the three pairs in a narrow groove, then the three in a wide groove, and so on; the two base pairs between grooves are masked and not seen. For the purpose of looking for symmetries, no distinction is made here between wide and narrow grooves, so half the possible castings become equivalent.

When this procedure was applied to the binding sites of *lac*¹ and *λ O_L1* (ref. 4), the result was unexpected. There was for each sequence, one "casting" which established two types of homologous features shared by the two repressor-binding sequences on one particular face of the helix (Fig. 1). These are: (1) a face sequence in each comprised of the same six "visible" base pairs with only the 5'-3' orientation reversed, and (2) a feature which, for lack of an established terminology, will here be called a G-C, A-T couplet; this is defined simply as a succession of three G-C base pairs preceded or followed, at a separation of seven base pairs, by a succession of three A-T base pairs, with the result that one wide groove filled by G-C

pairs would have an adjacent wide groove filled by A-T pairs in the helix.

As Fig. 1 shows, the 'upper' half of the *lac* sequence (positions 5-14), excluding backbone positions, is a perfect reflection of the lower part of the *λ* sequence (positions 17-26). As discussed below, this sequence is largely conserved in the *O_R1* (ref. 5) and *O_L2* (ref. 6) binding sites in that it forms part of the sequence which Maniatis *et al.* have noted to be repeated in the rightward and leftward *λ* repressor recognition sites. Considering *lac* and *O_L1* sequences alone, the random odds of such a match symmetrically located with respect to the centre of the sequences are less than 1 in 1,000. (The probability of a random match in a sequence of size six is $\sim (1/4)^6$ since the G-C content in *E. coli* and *λ* DNA is 51%. There are four chances for a match in the 4 halves of the two sequences. The probability of one or more such matches is then $1 - P(0)$ or $1 - [1 - (1/4)^6]^4 = 0.00097$.)

The second homologous feature shared by the *lac* and *O_L1* sequences as presented in Fig. 1 is what I have termed a G-C, A-T couplet in each sequence. The central wide groove contains only G-C pairs and the next lower wide groove contains only A-T pairs. The random odds of such matching couplets are $\sim 2(1/64)^2$ or 1 in 2,000. This feature, as noted below, also appears in *O_L2*, *O_R1*, *O_R2* and *O_R3*. Alignment of the *lac* and *O_L1* sequences, so that this feature is seen in the forward face, defines a helix face with considerable symmetry. For example, the symmetry of the *lac* sequence is still present in this representation. Most of the symmetry properties discussed here are limited to positions 4 to 24, noted by Gilbert and Maxam¹ to show substantial rotational symmetry about position 14 when viewed in the linear form. The two asymmetries previously observed in this rotation are split between the face sequence (10-18) and the backbone sequence (12-16); otherwise both face and backbone sequences still show rotational symmetry about position 14. It is interesting that the "correction" of the 12-16 asymmetry is a known constitutive mutation, and a correction of the 10-18 asymmetry would cause a recurrence in the lower half of the *lac* sequence of the common sequence shared by *lac* and *O_L1*. On the other hand, the symmetry seen in this view of the *O_L1* sequence was not suggested by Maniatis *et al.*⁴ but is clearly revealed by this format. In contrast to the *lac* sequence, the symmetry character of the *O_L1* sequence when viewed on this face of the helix is not a rotation but a reflection, in the face sequence, with seven of the ten face pairs reflected across the centre. This symmetry extends beyond the homologous regions picked out by Maniatis *et al.*⁶. The backbone sequence shows neither type of symmetry.

It is intriguing that this feature, the G-C, A-T couplet, appears in several other control sequences. The phage fd RNA polymerase binding site¹⁰ has such a couplet at its centre. The CAP sequence starts with two such couplets². One face in the promoter sequence of the *tyr* tRNA gene¹¹ shows three such couplets out of a possible four for that sequence size; in this case, however, it is a different face of the helix which shows perfect rotational symmetry centred on the rotational axis noted by Sekiya and Khorana¹¹ (here all asymmetries in the sequence fall on the backbone positions). The sequence for the attenuator region of the tryptophan operon¹² contains a successive series of six such G-C, A-T couplets in a row. This guarantees one such couplet on every face of the helix in the attenuator region.

In Fig. 2, the sequences of the *O_L2*, *O_R1*, *O_R2*, and *O_R3* binding sites^{5,6} have been aligned such that the G-C, A-T couplet is seen in each. The same alignment shows most of the *lac*-*O_L1* common sequence appearing in *O_L2* and *O_R1*; the one change is that T-A at position 22 in *O_L1* becomes A-T in *O_L2* and *O_R1*. This is the only change between *O_L1* and *O_R1* which falls within the recurring symmetrical 17-base-pair regions described by Maniatis *et al.*⁶ and is viewed by them as the probable cause of the weaker binding observed with *O_R1*.

The fact that the G-C, A-T couplet appeared in all these sequences and in several cases was associated with a recurring sequence suggested a search of the only other sequence known

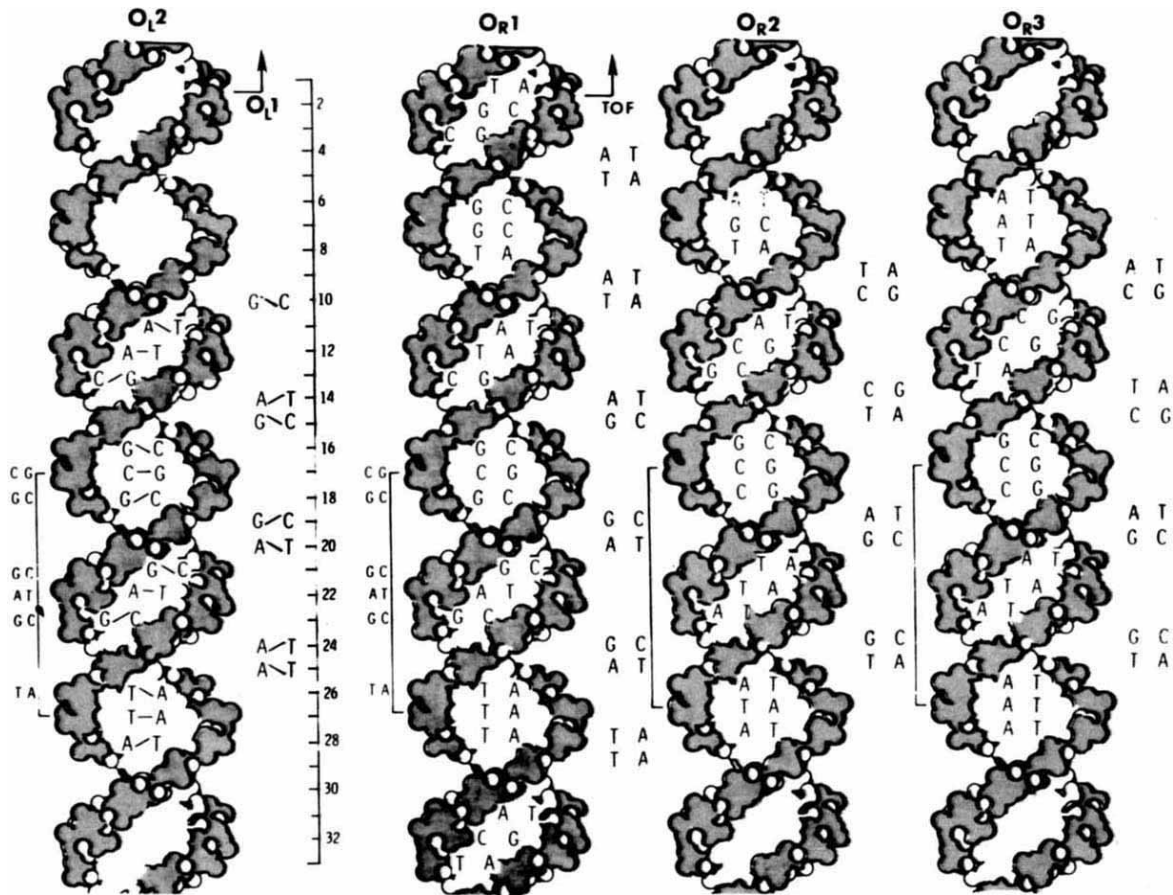


Fig. 2 The helices from left to right represent the repressor-binding sequences of O_L2 , O_R1 , O_R2 and O_R3 , respectively. The common face sequence shared by O_L1 , O_L2 , O_R1 and the *lac* repressor sequence is shown to the left of the O_L2 helix. The corresponding sequence in O_R1 is shown to the left of the O_R1 helix. The two base pairs hidden by the backbone on each turn are shown to the right of the respective helices. The brackets indicate the G-C-A-T couplet feature in each helical sequence. The dashed A-T at the top of the O_R2 sequence is the same pair shown at the bottom of the O_R1 sequence; the top pair in the O_R3 sequence is the next pair following the pair shown at the bottom of the O_R2 sequence. The sequences shown for O_R1 and O_R3 are on the same face of the helix, when viewed as a continuum; whereas the sequence shown for O_R2 is on a face rotated 108° relative to that shown for O_R1 and O_R3 .

to contain a repressor binding site, the 'leader region' of the tryptophan operon¹². As noted above, the attenuator region is rich in G-C,A-T couplets; there are four additional couplets preceding the attenuator region, the first appearing 13 bases from the starting point of transcription. But only the second G-C,A-T couplet in the attenuator region is preceded by a sequence similar to that seen in the *lac* and λ repressor-binding regions (Fig. 3). The recurring face sequence seen in these five cases is



The 5'-3' orientation of the sequence is the same in *trp* and *lac* with the reverse orientation seen in the three λ sites.

The chance occurrence of the juxtaposition of a G-C,A-T couplet with a very similar sequence in all these cases is very low. Neither feature nor the combination of the two, however, provides the symmetry one would expect for recognition by dimeric or tetrameric repressor molecules. The 17-base-pair groups with strong rotational symmetry, observed in the analysis of Maniatis *et al.*⁶, almost certainly serve this function. These are shown, in helical form, in Fig. 4. The faces shown here are rotated 36° relative to those shown in Figs 1 and 2 for O_L1 , O_L2 , and O_R1 , 72° for O_R2 and O_R3 . Thus the sequence similarities discussed above are seen on the face of the helix which is adjacent to the face in which a rotationally symmetrical group is seen in O_L1 , O_L2 and O_R1 . The symmetry groups themselves do not fall on a single face of the helix in either the rightward or leftward λ sites, for example, the face shown in

Fig. 3 Partial sequences from the *trp* attenuator region and the repressor binding sites of *lac*, O_L1 , O_L2 and O_R1 have been aligned by the position of their G-C,A-T couplets. The base pairs in bold letters represent the "common" sequence; those pairs in light letters are not part of this sequence but are part of the G-C,A-T couplets.

TRP	LAC	O_L1	O_L2	O_R1
A-T	T-A			
T-A	T-A			
G-C	G-C			
A-T	T-A			
G-C	G-C			
G-C	G-C	G-C	G-C	G-C
G-C	C-G	C-G	C-G	C-G
G-C	C-G	G-C	G-C	G-C
		G-C	G-C	G-C
		T-A	A-T	A-T
		G-C	G-C	G-C
A-T	A-T	T-A	T-A	T-A
A-T	A-T	A-T	T-A	T-A
A-T	A-T	A-T	A-T	T-A

Fig. 4 for O_L2 lies at 144° relative to that shown for O_L1 .

If the repressor-binding function is conceded to the rotationally symmetrical groups, what function can be suggested for the two recurrent features observed in this analysis? Furthermore, what mechanism would give rise to a sequence, shared, but with reversed $5' \rightarrow 3'$ orientation in different cases? A case can be made that the G-C, A-T couplet may be part of a recognition sequence for RNA polymerase. From its appearances in known control sequences, it seems that it may be involved in both promoter and terminator sequences. Though the probability of a G-C, A-T couplet occurring somewhere in a given promoter sequence is high (probability of one or more couplets for a sequence of size n is $1 - (31/32)^n \sim 13$ or 0.42 for an n of 30), the probability of finding it in 11 such sequences, often several times over, drops well below 1 in 100,000. It is true that this feature does not always show the same position relative to the starting point of transcription, but the relationship of the initial binding point to the starting point is unclear at this time^{6,13,14}. With regard to the terminator regions, the attenuator-terminated *trp* mRNA ends precisely with the last of the G-C, A-T couplets in the attenuator region¹². A possible terminator region for the *tyr* tRNA sequence reads CCATC---TTT-----CCC on the $5' \rightarrow 3'$ strand¹⁵. Here, as in O_L1 and O_R1 , ATC appears in the groove preceding a G-C, A-T couplet but the order of the A-T triplet and the G-C triplet is reversed.

How does the "common" sequence fit in? This sequence is juxtaposed with the G-C, A-T couplet only in repressor-binding regions (the claim of the *trp* attenuator region here is tenuous, however) and not in the promoters. The homologous sequence observed by Pribnow¹⁶ in six promoters appears in position 7 to 13 of O_L1 in Fig. 1, not in the common region. It is difficult

to see why there should be this sequence appearing with the same $5' \rightarrow 3'$ orientation in *trp* and *lac* and with the opposite orientation in O_L1 , O_L2 , and O_R1 . Another puzzling observation, the reflection symmetry seen on the O_L1 face in Fig. 1, may hint at an explanation. This symmetry, though definitely non-random (random odds $\sim 7/100,000$), would seem of little use to a repressor dimer. If the reflection symmetry of O_L1 were perfect, however, one would have a sequence containing the "common" sequence of the *lac* and *trp* orientation in the upper half and the "common" sequence of the O_L1 , O_L2 , and O_R1 orientation in the lower half and a G-C, A-T couplet in each direction. Divergence from a common precursor sequence of this type could produce the shared features observed here and might be related to those homologies observed by Pribnow as well. If this evolutionary hypothesis is correct and similarly obtains in eukaryotes, it could provide the molecular basis of an integrator gene¹⁶.

Consider the implications of this hypothesis for the *lac* and λ sequences. In *lac* it looks as though the common sequence is the recognition sequence for repressor; here the sequence has been duplicated in the lower half of the site (in place of the original reflected sequence?); in the λ sites it still forms parts of the basis of the recognition site but with a shift in reading frame to an adjacent face of the helix, preventing heterologous interactions. This view suggests that homologies would exist, as well, in the "dissimilar" halves of *lac* and O_L1 . If the upper half of the O_L1 sequence is compared with the lower half of the *lac* sequence after the latter has been rotated 180° , one observes the rather remarkable pattern that there is a perfect match at every other position in the two sequences, that is, *lac* position 23 = O_L1 position 8, *lac* position 21 = O_L1 position 10, and so on.

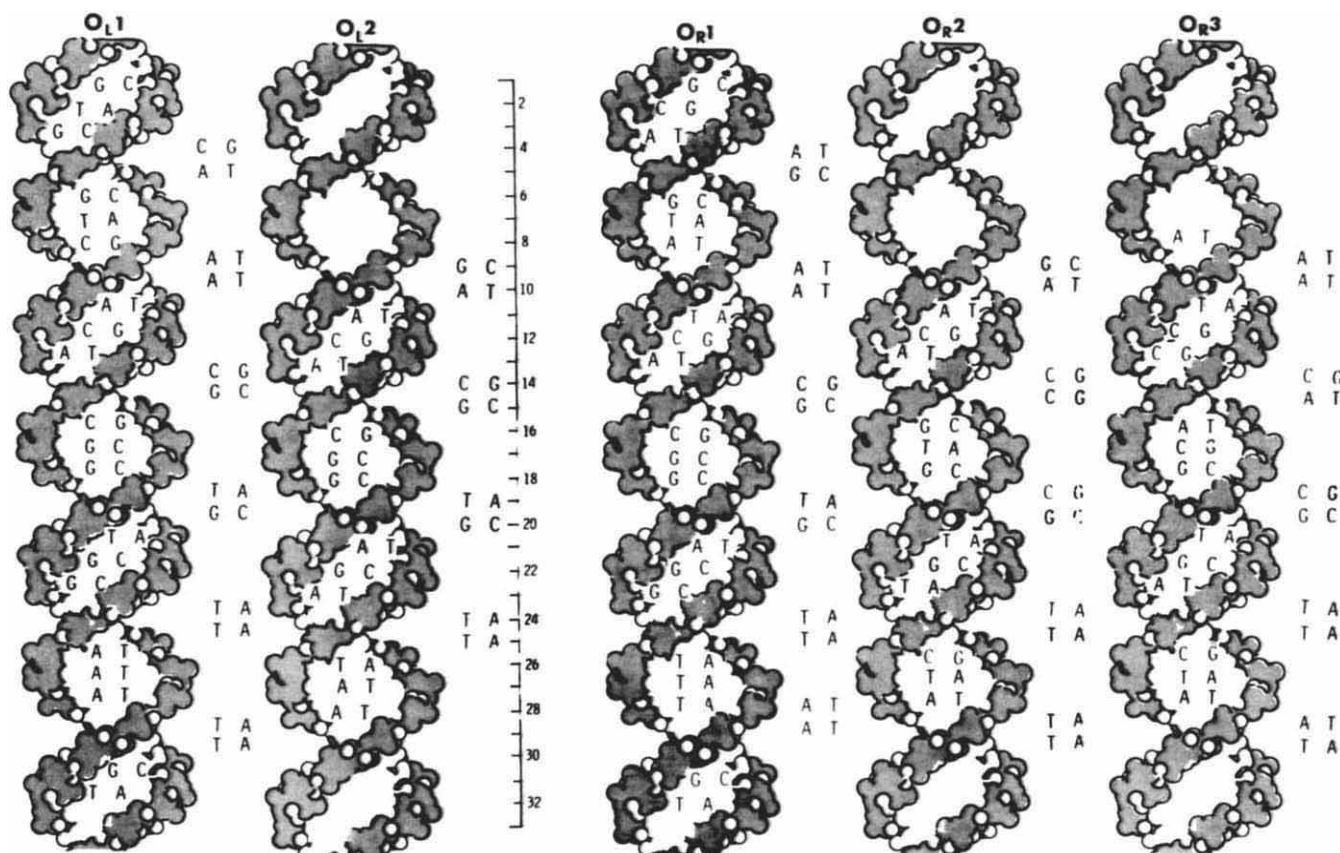
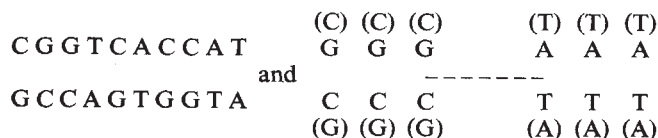


Fig. 4 In this view of the repressor-binding sequences of O_L1 , O_L2 , O_R1 , O_R2 and O_R3 , the rotational axis observed by Maniatis *et al.* has been located in the centre of the central wide groove in each case. The face shown here for O_L2 lies at a viewing angle of 144° relative to the face shown for O_L1 . The face shown for O_R2 lies at a viewing angle of 144° relative to the face shown for O_R1 ; that for O_R3 lies at 252° relative to O_R1 , 108° relative to O_R2 . O_L3 (not shown) lies on the same face as O_L2 . The sequences shown are contiguous; for example, the first base pair of O_L2 follows the last shown for O_L1 . The rotationally symmetric 17-base-pair groups of Maniatis *et al.* lie in positions 9 to 25. The O_L1 , O_L2 and O_R1 sequences have been moved upwards one position relative to Figs 1 and 2, whereas O_R2 and O_R3 have been shifted downwards two positions.

In conclusion, I suggest that synthetic DNA probes of the sequence



in both 5', 3' permutations may prove very useful, in addition to that proposed by Maniatis *et al.*⁶, in the investigation of promoters, terminators and repressor-binding sites.

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Detection of slow rotational motions of proteins by steady-state phosphorescence anisotropy

We have used the anisotropy of the phosphorescence from intrinsic chromophores of proteins to detect slow rotational motions of the macromolecules in highly viscous solutions. This method has potential for monitoring the mobility of proteins in structures such as membranes and viruses.

Rotational motions of the emitting molecules in a solution result in the depolarisation or loss in anisotropy of the emission. The quantitative relationship between the emission anisotropy observed in conditions of constant illumination and the rotational time is embodied in the Perrin equation¹

$$\frac{1}{A} = \frac{1}{A_0} \left(1 + \frac{\tau}{\theta} \right) \quad (1)$$

When the rotational correlation time θ is equal to the excited state lifetime τ , the anisotropy A decreases to half A_0 the anisotropy for a stationary emitter.

Weber applied emission polarisation² to the study of protein hydrodynamics using fluorescent labelled protein molecules. Fluorescence polarisation is widely used in probing the rotational mobility of proteins and the flexibility of their active sites in steady-state experiments³ and in nanosecond techniques⁴⁻⁶. Introduction of a fluorescent label is usually required in that the lifetimes of the intrinsic fluorescence from tryptophan and tyrosine residues are much shorter than the

rotational correlation times of most proteins. Rotational correlation times as long as 500 ns have been measured by the use of fluorescence probes with 100-200-ns excited singlet lifetimes⁷. Rotational motions which are much slower than the fluorescence lifetime, however, are difficult to detect.

For access to slower rotational motions a long lived emission is necessary. Whereas long lived phosphorescence is usually quenched in fluid solutions, we have shown that buried tryptophan residues in proteins which are protected from quenching by solvent-mediated processes, phosphoresce in fluid solutions with lifetimes in the millisecond range⁸. As a consequence, rotational motions occurring on this time scale and shorter become amenable to experimental detection. We report here a preliminary study on the use of steady-state phosphorescence anisotropy measurements to monitor rotational motions of proteins.

The tryptophan residues in proteins were excited at 300 nm from a mercury lamp. Phosphorescence was measured on the 0-0 vibronic transition of tryptophan ($\lambda = 410-415$ nm) which has the largest emission anisotropy^{9,10}. Measurements were obtained with a phosphoroscope described elsewhere¹¹, in which both the exciting and emission beams were polarised with Polaroid HNPs linear polarisers. Anisotropy A was calculated from the relationship

$$A = \frac{I_{11} - GI_1}{I_{11} + 2GI_1} \quad (2)$$

where I_{11} and I_1 are the emission intensities polarised parallel and perpendicular respectively to the vertically polarised exciting beam, and G is an instrumental correction factor¹². The values of A_0 reported are the largest values of A obtained at temperatures where the glass had softened somewhat eliminating strains and cracks, but molecular rotational motions were still insignificant.

The anisotropy of the tryptophan phosphorescence for several proteins and for indole in glycol-H₂O were determined. The values for the anisotropy of the 0-0 transition at temperatures below 120 K appear in Table 1. At these temperatures, near the glass transition temperatures of the solvents, molecular rotational times are longer than the 5-6-s tryptophan-triplet lifetime. These values of A_0 therefore, depend only on the electronic properties of the isolated chromophores and the extent of electronic coupling, or energy transfer, between chromophores. The connection between these limiting anisotropies and energy transfer between tryptophan residues in proteins in relation to earlier studies⁹, and to observations of heterogeneity in protein phosphorescence spectra¹³, will be considered elsewhere.

The rotational correlation time for spherical particles was

Table 1 Limiting phosphorescence anisotropy values A_0 and T_1 -values of some proteins in 1:1 propylene glycol-buffer

	A_0	P_0	Molecular weight	T_1 (°C)
Indole	-0.140	-0.22	117	-116
α -Chymotrypsin	-0.032	-0.05	23,000	—
DNase I	-0.095	-0.15	31,000	-96.5
BCA	-0.090	-0.14	31,000	-96.5
HSA	-0.140	-0.22	65,000	—
BSA	-0.140	-0.22	65,000	-93
LADH	-0.140	-0.22	80,000	-90
GBM	-0.102	-0.16		
S-13 viruses	-0.090	-0.14		
Erythrocyte ghosts	-0.083	-0.13		

P_0 , the limiting degree of polarisation, is included for comparison with earlier works where the old notation was used. The relationship between anisotropy A and the degree of polarisation P , used in the older notation, is given by

$$\frac{1}{A} = \frac{3}{2} \left(\frac{1}{P} - \frac{1}{3} \right)$$

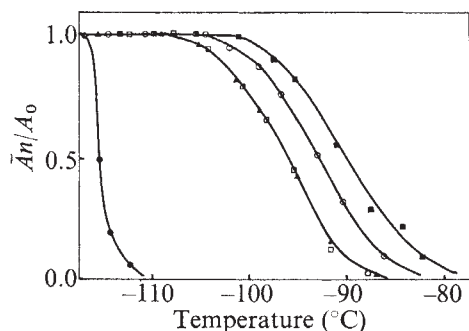


Fig. 1 Relative anisotropy of tryptophan phosphorescence as a function of temperature for globular proteins in 1:1 propylene glycol-phosphate buffer (0.05 M, pH 7). $\square$, DNase I (Worthington); $\blacktriangle$, bovine carbonic anhydrase (Sigma); $\circ$, bovine serum albumin (Worthington); $\blacksquare$, alcohol dehydrogenase from horse liver (Worthington). The anisotropy curve of indole ($\bullet$) in the same solvent is included for reference. All were at 10^{-4} M. In independent experiments A_n varied by less than 10%.

shown by Einstein¹⁴ to be equal to $v\eta/kT$ where v is the volume of the particle, η is the viscosity and k and T are the Boltzmann factor and temperature (K) respectively. In polar solvents at low temperatures there is a steep dependence of viscosity on temperature, so that the temperature dependence of the rotational correlation time reflects principally changes in viscosity. As the viscosity of the glycol-H₂O solvent decreases at higher temperatures, rotational motions of proteins manifest themselves with a decrease in the magnitude of A . Equation (1) shows that A becomes a measure of the relative mobility of various proteins if the lifetime remains constant. The triplet lifetimes, however, decrease at warmer temperatures and vary from protein to protein. Each of the anisotropy values, therefore, was corrected to the value A_n it would have at a fixed lifetime, arbitrarily taken as 3 s.

Figure 1 shows the variation of A_n with temperature for indole and for several proteins. The decline in phosphorescence anisotropy is observed at relatively low temperatures, or high viscosities. The temperature $T_{1/2}$ at which A_n/A_0 is reduced to one half is compared with the molecular weight of the protein in Table 1. As the volume of globular proteins increases with molecular weight, there is a concomitant increase in the temperature at which depolarisation occurs. The trend shown by molecular volumes compared with $T_{1/2}$ indicates that it is rotational diffusion of the entire macromolecule, rather than independent motions of the aromatic side chains, which is the dominating factor in depolarising the intrinsic phosphorescence of proteins. That the temperature-anisotropy profiles depicted

in Fig. 1 for various proteins reflect a viscosity-dependent process is confirmed in Fig. 2. As the solvent viscosity is increased from ethylene glycol-H₂O to propylene glycol-H₂O to glycerol-H₂O, there is a corresponding increase in the temperature range for which depolarisation occurs with a given protein. In addition, experiments (to be published) in which phosphorescence depolarisation is followed as a function of time at a given temperature, unambiguously establish rotational motions as the origin of these transitions.

When the present steady-state method of measuring phosphorescence anisotropies is used, the rotations of even relatively large proteins must be slowed down by means of high viscosities to observe the depolarisation. The technique suggests itself as a method for measuring rotational motions of larger macromolecular complexes or of proteins embedded in media of high viscosity where the relaxation times can be expected to be long. Figure 3 illustrates the feasibility of using phosphorescence anisotropy to monitor the rotational mobility of membrane and viral coat proteins. The emission anisotropy for proteins in rat glomerular basement membrane (GBM), human erythrocyte ghosts and the coat of S-13 bacteriophage are plotted as a function of temperature along with the data for indole.

In contrast with the behaviour of the soluble proteins and the

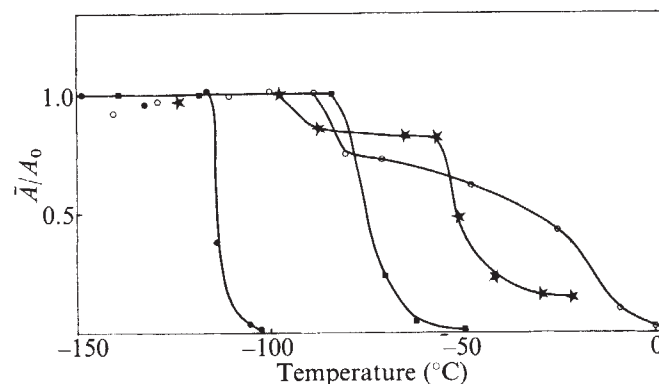
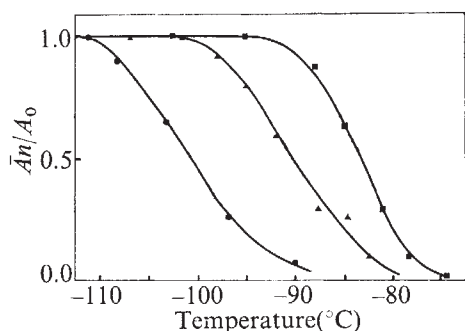


Fig. 3 Relative anisotropy of tryptophan phosphorescence as a function of temperature for biological structures suspended in propylene glycol-phosphate buffer (1:1). $\blacksquare$, GBM from rat kidney. They were prepared as previously described²³, in the laboratory of Dr N. Kalant at the Lady Davis Institute for Medical Research. $\star$, S-13 bacteriophage viruses, a gift of Dr J. H. Spencer, Department of Biochemistry, McGill University. The method for growth, collection and purification has been described before²⁴. $\circ$, Human erythrocyte ghosts were given by Dr Kahlenberg and were prepared by the method of Dodge *et al.*²⁵. With these suspensions the O₂ concentration of the solvent was reduced by bubbling the samples with highly purified N₂ (Union Carbide, Canada, 99.997% N₂) after which the 5-mm outer diameter, round, Spectrosil quartz sample cells were sealed. $\bullet$, Indole anisotropy curve in the same solvent is included for reference.

Fig. 2 Relative anisotropy of tryptophan phosphorescence as a function of temperature for alcohol dehydrogenase from horse liver in solvent mixtures of different viscosity. $\bullet$, Ethylene glycol-phosphate buffer (1:1); $\blacktriangle$, propylene glycol-phosphate buffer (1:1); $\blacksquare$, glycerol-phosphate buffer (2:1).



glycoprotein in GBM, the tryptophan phosphorescence of erythrocyte ghosts depolarises in stages rather than in a single transition. With increasing temperature an initial loss of anisotropy occurs at a viscosity at which soluble proteins (molecular weight 60,000–80,000) undergo depolarisation. The remaining anisotropy of the phosphorescence from the ghosts decreases slowly with increasing temperature. Only at much lower viscosity, about 0 °C, is most of the anisotropy lost. The data suggest that some proteins are highly constrained through interactions between macromolecular components although others are relatively free to undergo independent rotational motions, and probably correspond to specific types of membrane proteins at the surface. Selective removal of particular proteins¹⁵ followed by phosphorescence measurements would establish the mobility of various membrane

proteins and contribute to the elucidation of their role in membrane structure.

There is a similar discontinuous temperature profile of the phosphorescence anisotropy of the coat proteins of S-13 bacteriophage. The data establish that in the sample investigated, several rotational correlation times are involved. While the results may reflect some independent motion of the individual coat proteins in the virus capsule, due to the rather harsh deoxygenation treatment of this sample (bubbling with N_2) the observed heterogeneity probably arises from damaged and aggregated phage particles. The depolarisation profile is included to reflect the ability of the technique to detect heterogeneous rotational correlation times and to follow the motion of large macromolecular complexes. The anisotropy-temperature profile for GBM from rat kidney reveals that tryptophans are not rigidly fixed within the structure in that loss of anisotropy is observed at a temperature characteristic of soluble proteins (molecular weight $\sim 100,000$). GBM is composed primarily of collagen and one or more glycoproteins¹⁶. The glycoproteins therefore cannot be rigidly fixed within the insoluble basement membrane matrix but must have considerable rotational mobility of their own.

Several features of this technique for monitoring rotational motions of proteins are noteworthy. (1) Rotational motions are detected by the use of the depolarisation of the emission from intrinsic chromophores in proteins, thus avoiding labelling of proteins with probes. There are no concerns about the extent of perturbation to the system and the possibility of independent motions of the labelling group itself. A perturbation has been introduced instead with the use of glycol-water solvents. Studies of the biological activity^{17,18} and the X-ray analysis^{19,20} of proteins in these solvents, however, suggest that the perturbation is small.

(2) In the investigation of more complex systems involving two or more proteins, such as with the erythrocyte ghost membranes, the use of the intrinsic emission offers the possibility of resolving the rotational correlation time into contributions from individual proteins. Selective observation of particular proteins is also possible. With structures such as GBM the tryptophan phosphorescence arises only from the glycoprotein component²¹ in that tryptophan is generally absent from collagen²².

(3) The method is useful for investigating slow rotational motions. The functioning of proteins not only as soluble cellular entities but within macromolecular complexes and in insoluble structures, such as membranes and muscle fibres, makes necessary the development of techniques to elucidate the structure and dynamics of such systems. We feel that phosphorescence anisotropy measurements can contribute to the understanding of the molecular basis of the function of such systems.

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Errata

In the article "Eukaryotic DNA replication complex" by V. M. Genta, D. G. Kaufman, W. K. Kaufman and B. I. Gerwin (*Nature*, **259**, 502; 1976) in the last paragraph, lines 8-13 should read . . . the observation of ATP-dependent DNA synthesis in M-band fractions isolated from sea urchin embryo nuclei¹⁴. Since nuclear membrane is largely confined to the LB (unpublished observation), the result reported here differs from studies involving the M-band technique¹⁴⁻¹⁶ because . . .

In the article "Dissociation of response to injected gonadotropin between the Graafian follicle and oocyte in pigs" by R. H. F. Hunter, B. Cook and T. G. Baker (*Nature*, **260**, 157; 1976) the first entry in the 7th column of Table 1 should read 81.2% and not as printed.

In the article "Gibberillic acid enhances the level of translatable mRNA for α -amylase in barley aleurone layers" by T. J. V. Higgins, J. A. Zwar and J. V. Jacobsen (*Nature*, **260**, 166; 1976) Figs 1 and 3 have been transposed. The legends are correct as they stand.

In the article "New H₂O celestial sources associated with H II regions in the Southern Hemisphere" by P. Kaufmann *et al.* (*Nature*, **260**, 306; 1976) the second entry in the 1st column of Table 1 should read 330.9-0.4 and not as printed.

In the article "Effect of surface roughness on rolling friction and adhesion between elastic solids" by G. A. D. Briggs and B. J. Briscoe (*Nature*, **260**, 313; 1976) the first line under the equation in the first paragraph should read: where σ is the distribution of asperity heights . . .

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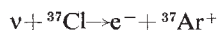
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matters arising

Polymerisation and the solar neutrino problem

JACOBS¹ has questioned the efficiency of the helium flushing method for extracting Ar⁺ ions produced in the reaction



He suggests that Ar⁺ ions could induce polymerisation in the liquid C₂Cl₄ and get trapped before they are neutralised by capturing electrons, and consequently all the Ar⁺ ions produced in the above reaction are not detected.

Tetrachloroethylene is an unsaturated molecule which undergoes polymerisation, when irradiated by MeV γ rays, at the rate of $\sim 2.1 \times 10^{-10}$ mol ml⁻¹ s⁻¹ (based on the dose rate of 1.5×10 eV ml⁻¹ s⁻¹ and the polymer yield in the radiation experiments)². It is known that halogenated ethylenes undergo predominantly free-radical polymerisation, the cationic initiation of the chain is less likely because of the presence of electronegative groups in C₂Cl₄ leaving an electropositive carbon atom at the polymerisation site. Even if Ar⁺ can initiate polymerisation, the rate will be much smaller than that quoted above for γ rays. On the other hand, since the electron affinity of Ar⁺ is high, the Ar⁺ radical produced by the solar neutrino can be neutralised at a very fast rate. The electron transfer between the neutral radical and the positively charged ion is diffusion controlled with the rate constant 10^9 – 10^{10} l mol⁻¹ s⁻¹, while the rate constants of generalised polymerisation³ are of the order of 1–30 l mol⁻¹ s⁻¹. It is clear there will be negligible trapping of Ar⁺ ions through polymerisation in the tank.

The foregoing arguments receive indirect but unambiguous support from two experiments: (1) Barabanov *et al.*⁴ have measured the relative efficiency of removing a small number of ³⁷Ar atoms by helium purging in liquid C₂Cl₄ and in solid C₆Cl₆ by irradiating both the substances with fast neutrons from a PuBe source. They find that for the same number of fast neutrons, the same number of ³⁷Ar atoms is produced. Considering the different physical and chemical properties of C₂Cl₄ and C₆Cl₆, the probability of identical rate of trapping is surely negligible. (2) In the helium-purging efficiency experiment of Davis⁵, liquid C₂Cl₄ was irradiated with neutrons from an RaBe source to get an apparent ³⁷Ar production rate per incident neutron of 7.5×10^{-7} . If chemical trapping is im-

portant, the true production rate should be at least an order of magnitude higher than the observed one. The cross sections for the nuclear reactions



are sufficiently well known to render a theoretical calculation of the true production rate of ³⁷Ar possible; we find it to be $\sim 4 \times 10^{-7}$, in reasonable agreement with Davis' measurements.

We may add to these the very careful analyses by Wolfendale *et al.*⁶ and by Cassidy⁷ of the cosmic ray-induced background in Davis' experiment. These analyses lead to values for the photo-nuclear cross section which are in substantial accord with other cosmic ray and accelerator measurements. If the normalisation of the background at the depth of 25 m.w.e. (metres water equivalent) is wrong as implied by Jacobs, this would hardly have been the case. The conclusion thus seems inescapable, that chemical trapping of any kind—polymerisation or other chemical process—is of negligible significance in Davis' experiment.

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JACOBS REPLIES—In a brief communication¹ I explored the logic of the possibility that the solar neutrino problem might be caused by the chemistry of the solar neutrino experiment. I concluded that this chemical hypothesis was viable only if ³⁷Ar⁺ became 'chemically trapped' in Davis' system; five mechanisms for trapping the argon in C₂Cl₄ were suggested.

Banerjee *et al.*² criticise one of my trapping mechanisms—induced polymerisation in the liquid tetrachloroethylene—and imply by heuristic arguments that Davis' neutron-irradiation test³ and the cosmic-ray background test^{4,5} completely rule out the possibility of any significant

'chemical trapping'. I suggest that the conclusions of Banerjee *et al.* are comfortably plausible, but they are by no means logically inescapable.

Induced polymerisation of C₂Cl₄ is the least tenable of my suggested trapping mechanisms; the large atomic radius of chlorine will hinder the formation of any long chain polymer¹. It is much more probable that the argon is 'chemically trapped' in a small bound molecule or ion-molecule. One could just as easily interpret the results of Barabanov *et al.*⁴ as positive support for the formation of ArCl_n, with $n = 2, 4$ or 6 .

Order-of-magnitude arguments are unsafe in this controversy. The uncertainties intrinsic to present-day solar models can easily render acceptable a theoretical solar neutrino rate as low as 4 SNU (ref. 7), whereas Davis' latest two runs (36 and 37) indicate an experimental rate of ~ 4 SNU—with a 1σ upper limit of ~ 1.5 SNU for the average of runs 21–37 (ref. 7; estimated by eye from their Fig. 2). Thus, 'chemical trapping' need not hide 9 of every 10 argon atoms produced by solar neutrinos, but could well conceal only 60% of the argon on average.

Therefore, order-of-magnitude (or even crude qualitative) agreement between theory and experiment in some of the crucial tests^{3–5} is not sufficient to eliminate the chemical hypothesis—only precise quantitative matching can do that. Finally, I conclude again that my chemical solution to the solar neutrino problem remains a viable alternative.

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Sub-microscopy porosity and superfluid ⁴He

VAN Brakel and Heertjes¹ have reported on transfer phenomena in porous media, using the capillary rise of a wetting liquid. I would like to add to this by mentioning a phenomenon apparently similar to capillarity, although funda-

mentally different: the behaviour of superfluid ^4He in narrow gaps. This might contribute to the knowledge of transfer phenomena.

The behaviour of superfluids is not as well known as capillarity of a normal viscous wetting liquid. The viscosity of superfluid helium is zero. This provides a method² to detect and to estimate the size of sub-microscopic pores in solid materials as well as in small, packed grains. Those materials with small enough pores and through which only the superfluid helium flows are called superleaks.

When liquid ^4He is cooled to a temperature of 2.17 K, the λ point, there is a phase transition, and some of the liquid becomes superfluid. As the temperature decreases further, the proportion of superfluid increases. The temperature at which the superfluid starts to flow through a superleak is called the onset temperature and is always below the transition temperature. The difference between the two temperatures is the 'depression of the transition temperature'. The smaller the pores the lower the onset temperatures.

The channels through a superleak are not uniform but are at random, interconnecting each other. If ϕ_m is the minimum diameter along a connection path, then the largest ϕ_m of all possible paths determines the onset temperature, because in that diameter superfluid movement will occur first, that is, at the highest temperature below the λ transition. This is a 'size effect' and arises from interactions between excitations of quasi particles of the liquid helium and the solid. When the temperature is high and the gap or diameter is small, the interaction is equivalent to the presence of viscosity. The number of excitations diminishes with the temperature.

To estimate the widths of the gaps, or the 'diameters' of the pores through which the superfluid penetrates the superleaks, the following two relationships between the depression ΔT_λ of the transition temperature and the width d of those gaps are used

$$\Delta T_\lambda \simeq - \frac{2 \times 10^{-14}}{d^2} \text{ K}$$

$$\text{or } \Delta T_\lambda \simeq - \frac{2.5 \times 10^{-11}}{d^{3/2}} \text{ K}$$

if d is expressed in cm. The first relationship was derived by Ginzburg and Pitaevskii³ and the second, which seems to be more consistent with the experimental data, by Mamaladze⁴. It was, however, pointed out⁵ that the width of a parallel-sided channel, which probably occurs most frequently, is $2d$. Using this latter criterion and the two expressions

given above, the width of the channels in a granite and a limestone, which were found to be superleaks, was estimated for both between 34 Å and 22 Å (ref. 6) and the onset temperatures measured were 1.48 K and 1.45 K, respectively.

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Antibiotic resistance in laboratory workers

RECENT publications by Williams Smith¹ and Anderson² have been concerned with the ability of *Escherichia coli* K12 to survive in the human alimentary tract. This is a matter of importance since a

containing either ampicillin (25 µg ml⁻¹), streptomycin (10 µg ml⁻¹), tetracycline (25 µg ml⁻¹), chloramphenicol (25 µg ml⁻¹) or nalidixic acid (30 µg ml⁻¹). Resistant colonies arising on these plates (if any) were then tested for indole production in peptone water, and for acid+gas production in McConkey broth after overnight incubation at 44 °C (refs 3 and 4). If available, ten resistant colonies from each plate were O-antigen typed⁵ and tested for the transferability of their resistance patterns by mating with a standard *E. coli* R⁻ (ref. 5). In all, 590 isolates were examined in this way.

A total of 38 R-plasmid carrying *E. coli* have been isolated so far, and seven different resistance patterns have been encountered (Table 1). None of these resistant *E. coli* was UB1139, nor were the resistance patterns encountered those of the plasmids used for experimental purposes.

The workers concerned in these studies used conventional procedures for testing low risk bacteria^{6,7}. Mouth pipetting was not used but experiments were conducted in the open laboratory. These results therefore reinforce the views of Williams-Smith¹ and Anderson² that *E. coli* K12 lines are unlikely to establish themselves

Table 1 Comparison of the resistance patterns of the plasmids isolated from faecal samples with the patterns of the experimental plasmids used by the workers concerned

Faecal samples		Experimental plasmids	
Pattern	No. of times isolated	Plasmid	Pattern
Tc	23	Rldrd19.K1	Kn
Sm Su	3	R46	Tc Sm Sp Ap Su
Tc Su	1	R55-1	Su Cm
Sm Ap Su	1	R64-11	Tc Cm
Tc Sm Su	8	R100-1	Tc Sm Su Cm
Tc Sm Ap Su	1	R388	Su Tp
Tc Sm Ap Su Cm	1	R391	Kn
		R751	Tp

Total number of isolates examined: 590. Abbreviations for resistance markers: Tc, tetracycline; Sm, streptomycin; Ap, ampicillin; Su, sulphonamide; Cm, chloramphenicol; Sp, spectinomycin; Kn, kanamycin; Tp, trimethoprim.

detailed knowledge of the behaviour of this much used *E. coli* strain is needed to assess the potential hazard of many genetic engineering experiments.

In this department we have monitored the faecal flora of laboratory workers engaged in work with R-plasmids to see whether they have ever excreted the K12 strains with which they were working, or other *E. coli* carrying plasmids which were being studied in this laboratory. Most of our experiments have involved a restricted number of R-plasmids (Table 1) in *E. coli* UB1139, a nalidixic acid-resistant derivative of *E. coli* K12.

A total of 52 samples was taken from the two individuals under study during a period of 184 d. Suspensions of faecal material in physiological saline were plated on McConkey agar to determine the total coliform count, and on agar

as major components of the faecal flora of workers handling these bacteria so long as reasonable laboratory procedures are used.

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reviews

Dinosaur teleology

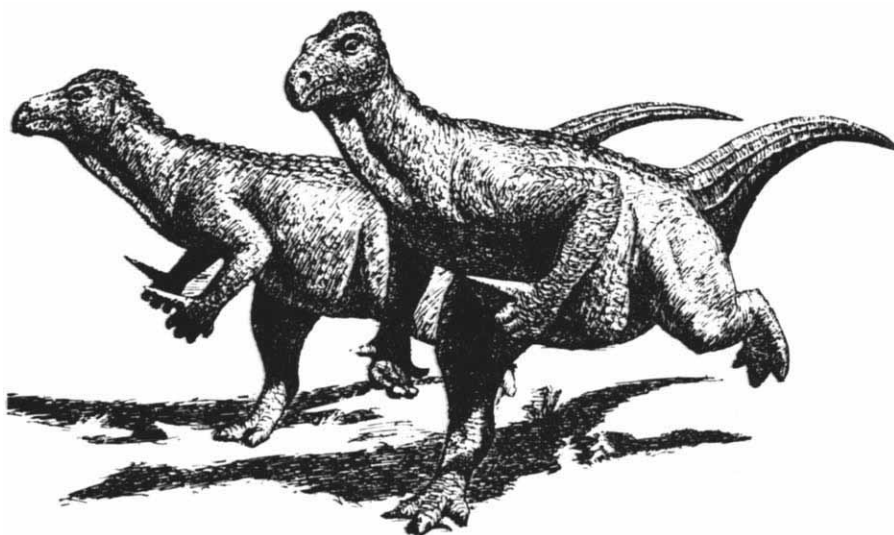
L. B. Halstead

FOR 140 million years the dinosaurs dominated the continents, then 64 million years ago they suddenly disappeared. These animals have always been accepted as the acme of the world of cold-blooded reptiles. In recent years, however, R. T. Bakker has been arguing that their former success was a consequence of their high metabolism and fast energy production (*Nature*, **238**, 81–85, 1972). He considered that they were warm-blooded in the same sense as birds and mammals, maintaining a constant internal temperature (homoiothermy) by means of their own internal activity (endothermy). Adrian Desmond's *Hot-Blooded Dinosaurs** is a fascinating account of the history of the study of dinosaurs and the changing views of their physiology, culminating in a vigorous exposition of Bakker's ideas. As an exercise in evangelism it is exemplary.

Desmond uses the old legal trick of the leading question. How could *Tyrannosaurus* have engaged in lengthy gladiatorial combats with *Triceratops*, if they had not had a high metabolic rate? How could *Struthiomimus*—the ostrich dinosaur—have maintained a speed of 80 km h^{-1} with the physiology and energy output of a lizard? The answers quite clearly are that they could not; but what Desmond fails to establish is that such behaviour actually took place or was even necessary.

All the evidence marshalled to support the notion of dinosaur endothermy is capable of alternative interpretations. The stance and gait of dinosaurs with the limbs positioned beneath the body is comparable to that found in birds and mammals. This stance seems to be associated with endothermy today. There is therefore a *prima facie* case that the energy requirements may have been the same; yet the crocodile's high walk is not evidence for endothermy—there is an evidence in an upright posture *per se*.

Much emphasis has been made of the



different predator-prey ratios between living ectothermic and endothermic populations. If one examines mammalian ratios of kg predator/kg prey, it can be seen that this changes from small animals to larger. The 'endothermic' situation with the dinosaurs could be interpreted as a direct consequence of their large size.

Perhaps the most striking similarity between dinosaurs and mammals is found in the microstructure of their bone. The laminar bone of sauropod dinosaurs is indistinguishable from that of some of the larger artiodactyls and it is presumed that this indicated a similar metabolism. Such microarchitecture may in fact be simply due to the rate of bone formation and is thus characteristic merely of large animals. The large dicynodonts with their sprawling gait (and therefore not comparable in terms of their locomotion to the dinosaurs) have the identical bone histology. The evidence for endothermy from bone histology is not particularly convincing.

The existence of dinosaurs in latitudes that were situated in the Cretaceous Arctic would seem at first glance to be strong evidence for endothermy. To speak of the Arctic at the time of the dinosaurs is a little misleading as there were no polar ice caps. The temperature of the Cretaceous seas at differing latitudes has been ascertained from oxygen isotope studies and the mean sea level air temperatures subsequently calculated. The range from Equator to Arctic was $31\text{--}14^\circ\text{C}$. It is

not unreasonable to imagine the odd dinosaur surviving such conditions.

The final piece of argument concerns the first bird *Archaeopteryx* and the pterosaurs. Both types of animal originated from the same basic stock and the birds seem to be directly descended from the dinosaurs. Birds and pterosaurs had an insulating covering as did the advanced paramammals and primitive mammals. In all these groups, it is accepted that they possessed a high metabolic rate and were genuine endotherms. Desmond contends that as *Archaeopteryx* was skeletally similar to small theropod dinosaurs and possessed feathers primarily for insulation, then perhaps such a covering was a common feature of all these animals.

Once the premise that dinosaurs must have been endotherms is accepted, it is but a short step to remove them from the Reptilia and place them in a new class, the Dinosauria, which includes the birds Aves (Bakker and Galton, *Nature*, **248**, 168–172, 1974). Desmond for his part proposes the new super-class Endosauropsida to encompass the classes Dinosauria (including Aves) and Pterosauria.

The case for dinosaur endothermy has certainly not yet been convincingly established and, even if it were, there would still be no justification for such a drastic restructuring of the classification of the vertebrates. Even the man in the street has some inkling of what a bird is and in no sense does this equate with dinosaurs. Brontosaurus and

**The Hot-Blooded Dinosaurs: A Revolution in Palaeontology*. By Adrian J. Desmond. Pp.238. (Blond and Briggs: London, November 1975.) £5.95.

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blackbirds have precious little in common beyond sharing an ultimate common ancestor. The evolutionary line of the mammals can be traced back to the paramammals, but this is not a sound basis for terming the mammal-like reptiles 'mammals', nor for including the mammals in the Paramammalia. The fact that we can now begin to indicate lines of descent is not a licence to redraft the classification.

The conclusions drawn by Desmond run far ahead of the available evidence. A great deal more work will be required before such notions of dinosaur endothermy can be taken seriously. There can be little doubt, however, that the dinosaurs were warm-blooded. The dinosaurs achieved a passive type of homoiothermy by being large; their surface-volume ratio would have ensured a fairly constant internal temperature. All the features that have been used to attribute endothermy to the dinosaurs can more easily be interpreted as being a direct consequence of their size.

It is a pity that Desmond's undoubted literary talents were not used in a more deserving cause than promoting hot-blooded dinosaurs. □

Conceptual shorthand

Transport Phenomena in Medicine and Biology. By M. Min-Shing Lih. Pp. xviii+531. (Wiley-Interscience: New York and London, 1975.) £14.70.

THIS book gives a very clear presentation of the mathematics necessary to describe—with a fair degree of rigour—the underlying physics involved in physiological processes concerned with transport of mass, momentum and energy. The author is to be complimented on achieving his declared aim of demonstrating how the mathematical conceptual 'shorthand' of physics can reduce the apparent phenomenological diversity of physiology. From the start the emphasis is on translation from the real world to the mathematical model, solution of the problem and back to the real world to present the answer in physical terms. The mathematics is continuously related to reality so that an experimentalist has his interest maintained and is carried along with the argument. This is done in a direct and refreshingly conversational way, producing more the flavour of a verbatim record of a colloquium than a concise university text.

With such a discursive style the author does well to contain his subject within 500 pages. The first five chapters present the mathematics necessary for a proper description of material processes in terms of con-

Synaptic receptors

Synaptic Receptors: Isolation and Molecular Biology. (Modern Pharmacology-Toxicology: A Series of Monographs and Textbooks.) By Eduardo de Robertis. Pp. xiv+387. (Dekker: New York, 1975.) \$29.50.

IN those synapses with chemical transmission, the transmitter substance acts on the postsynaptic membrane by combination with synaptic receptors. Within the last ten years, our knowledge of these receptors has advanced from a condition in which their existence as discrete structures was far from established to a situation in which several have been isolated. Their isolation is the subject of Dr de Robertis' book.

Synaptic receptors, as constituents of membranes, are amphipathic and there are, broadly speaking, two approaches to their isolation. The first uses detergents in aqueous solution to counteract their hydrophobicity and effect solubilisation. The second approach seeks to take advantage of this hydrophobicity by extracting receptors into non-aqueous solvents of low polarity. Dr de Robertis has played a dominant role in the development of the latter

approach, whereas most other work has concentrated on the former. Recently, Barrantes *et al.* (*Nature*, **256**, 325-327, 1975) have shown that, at least in the case of the acetylcholine receptor, there is nothing in common between the material isolated by these two methods. This casts a long shadow over the book, for in spite of a superficial balance, it is, in fact, an apologia for Dr de Robertis. This should surprise no one for Dr de Robertis' prolificacy is truly remarkable, having isolated some half dozen receptors from twice as many tissues.

The need for an expensive monograph at the present time is open to the strongest doubt, more so since Dr de Robertis has displayed the virtue of brevity in a subsequent review covering the same ground (*Rev. Physiol. Biochem. Pharmac.*, **73**, 9-38; 1975). The book contains a quite incredible number of typographical errors from the merely irritating to the downright misleading.

Until such time as our knowledge of synaptic receptors enables distinction between the permanently valuable and the ephemeral, a book of this kind is prey to premature obsolescence and, at the price, just not worth it.

David Green

tinuum physics. Chapters 6, 7 and 8 show us how to apply the mathematics to changing conditions as diverse as pulsatile blood flow, body heat loss, placenta mass transport and gastric acid analysis. Chapters 9 and 10 are specifically dedicated to haemorrhology—the non-Newtonian behaviour of blood as a suspension of cells flowing through narrow vessels—and oxygen transport. This last chapter is an impressive discussion of a vital process, including examples of varied problem solving from membrane blood oxygenators to the oxygen-tension profile in the cornea.

We are told that the original manuscript was 'test-used' in a Chataqua-type programme, and I would agree that the final book is very well suited for this self-help with guidance type of learning. The comprehensive presentation of 'Mathematical Background' in Chapter 2 makes the book self-contained. The reader is taken from functions and variables (p16) through to the tensor and its operations (p96), and by this time is ready to accept quite naturally the need for a tensor as a description of the stress on an element of fluid. I particularly liked the use of small print flow diagrams to illustrate on one page a single application of the mathematics being discussed. All of the mathematical development is integrated with the rest of the book through the use of

problems.

Because of the excellent integration I wondered if it might not be difficult to easily 'dip' and use parts of the book as a companion text in an undergraduate course—say haemodynamics. For example, the pursuit of generality in the development of the mathematical models means that to arrive at a particular point such as the description of pulsatile flow in a pipe seemed to be longer than Womersley's direct approach. The very applied flavour, however, of the maths makes the attempt an attractive/worthwhile proposition. Certainly I have not found any more suitable text for that type of course.

Some of the material in this book will be familiar to any teacher in this field and even the organisation may in part be already in use. Yet the whole achievement of keeping the practical biomedical quality of the mathematics always so apparent makes this a most welcome book. It will certainly be a great help in courses at final special honours or MSc level in this country. Although the fly-leaf suggests it is an ideal text for the student I do not believe that it is an easy book for the unaided beginner. For the postgraduate student and the biomedical researcher it should handsomely repay the investment of time necessary to come to terms with this very able and challenging presentation.

R. G. Gosling

Chemical Fortran

Fortran IV in Chemistry: An Introduction to Computer-assisted Methods. By G. Beech. Pp. x+303 (Wiley: London and New York, October 1975.) £8.75.

UNDERGRADUATE chemistry courses have so far not shown as much response to the advent of the computer as engineering courses, and they do not reflect its importance as a research tool. It is still possible to teach both theory and laboratory techniques without reference to the computer; the main impetus towards Fortran programming and computer-assisted techniques lies in the knowledge that nearly every chemistry graduate soon becomes involved with computers. Dr Beech's book will be invaluable to those responsible for the introduction of these methods at undergraduate level, and essential reading to undergraduates and research students meeting them for the first time. Perhaps the most striking feature of the book is the care with which

scopical data (including curve addition and reduction), crystal field simulation the chemistry and the underlying mathematics are developed as well as the computing. The operation of the programs listed is always fully described in the text.

An elementary knowledge of Fortran is assumed. The first chapter gives those elements not met on a first programming course, and those which may depend on the particular machine used. Next, normal numerical and statistical methods are given, including non-linear equations and a particularly clear account of matrix operations and eigenvalues. Practical examples of the application to experimental data are taken from chemical kinetics, electrochemistry, thermal analysis, multi-component spectroscopic data and mass spectroscopy. The next chapter covers a very wide range of computer simulations of experimental observables: modelling simple kinetic and radiochemical systems (including Monte Carlo techniques), thermodynamics and equilibria, crystal lattice

energies, statistical mechanics, spectroscopy and nuclear magnetic resonance line splitting and intensity. Theoretical topics treated include particle-in-a-box (one dimensional with bond length alteration, and two dimensional) and atomic and molecular orbitals (LCAO and Huckel calculations). In this section, emphasis is rightly placed on plotting out the results.

Data acquisition by analogue to digital instruments is described, followed by data processing (including convolution smoothing and baseline corrections), illustrated by application to nuclear magnetic resonance spectra. Complex data systems are described for mass spectrometry, gas chromatography and γ -ray spectrometry. The book closes with a useful review of the literature, including handling chemical information on the computer. Each chapter is followed by problems and a bibliography. The book is well produced, and in view of its extensive coverage, good value. It is warmly recommended for both undergraduate and postgraduate use. **B. P. Levitt**

Changing role of ion beams

New Uses of Ion Accelerators. Edited by J. F. Ziegler. Pp. xii+482. (Plenum: New York and London, 1975.) \$33.60.

THE current state of world economics demands that equipment, manpower and money are used as efficiently as possible. Nuclear physics has long been singled out as one of the 'big science' areas and more than a generation of expansion has provided a legacy of ion accelerators of which the low energy machines (that is about 5 MV and below) are used increasingly today in fields far removed from the compilation of nuclear structure data. But the story now is not merely one of keeping highly specialised machinery occupied. So interesting and valuable have the applications of ion beams to materials science and solid-state physics turned out to be that many Van de Graaff accelerators, for example, are now used exclusively in analytical experiments complementing (or replacing) more well established techniques. An analogous situation exists in the new field of ion implantation where exciting property changes are induced with ions usually below 500 keV in a variety of materials. The result is that nuclear physics laboratories are undergoing a metamorphosis towards solid-state applications which encompass physics, chemistry, materials science and metallurgy.

This book covers new developments

in analytical methods, such as Rutherford backscattering, channelling, nuclear reactions and characteristic X rays for composition analysis, as well as ion implantation and ion-induced X rays. The editor deserves praise for welding the wide-ranging subject matter so satisfactorily; and it is perhaps significant that nearly four-fifths of the book relates to analytical techniques since this is the area in which ion accelerators are making immediate impact. The previous volume—which had a similar title—appeared eight years ago. It was an excellent monograph which should have been more widely disseminated and its publication set the stage for constructive interaction between accelerator users and other physicists. It is encouraging to see how much ground has been covered in the intervening period and it is interesting to note the change of emphasis. In the present volume, for example, nuclear astrophysics and beam foil spectroscopy give way to superconductivity, corrosion studies and pollution species detection.

The principal value of this book is the bringing together of the detailed procedures for backscattering and X-ray data collection and analysis, as well as up-to-date overviews of current developments in ion implantation and X-ray excitation theory. It is a rare thing to find accounts written from what is clearly first-hand experience of the systematics of establishing a first-class analytical service using an accelerated beam of protons or helium ions. Inevitably in a book consisting of contributed chapters there is duplica-

tion but the essentials are there, and numerous real-life examples are included. Surprisingly there is very little reference to the ion implantation of semiconductors. Perhaps it was felt that this important field (virtually synonymous now with advance in device technology) receives adequate cover elsewhere. As semiconductor implantation, however, has provided the bread and butter of the industrial side of ion implantation for many years, and is likely to continue doing so, an omission of this sort is regrettable. It is unfortunately made all the more obvious since the contributed chapter on superconductors is wrongly labelled 'semiconductors' in the text! Rutherford backscattering should be referred to as such to distinguish elastic Coulomb interactions from inelastic reactions arising within the nucleus (nuclear backscattering). Although they receive attention in separate chapters, the two are referred to a little indiscriminately elsewhere. Having said that, the treatment and power of such methods of analysis (and the two chapters on ion-induced X-ray physics) are excellently covered.

Accelerator users, present and intending, should reach for this volume. No-one contemplating an integrated analytical programme or materials project with solids should be ignorant of the applications of ion beam machines and the growing expertise which surrounds them. It is a pity that oversights in production and a lack of standardisation of diagrams detract slightly from an otherwise well conceived book.

N. E. W. Hartley

Spin labelling

Spin Labelling: Theory and Applications. (Molecular Biology: An International Series of Monographs and Textbooks.) Edited by Lawrence J. Berliner. Pp. xiii+592. (Academic: New York and London, January 1976.) \$49.50; £25.55.

SPIN labelling is an obviously attractive technique. It is relatively easy to attach a spin label to a protein or to insert it into a membrane to act as a reporter group. Furthermore, the electron spin resonance spectra obtained are often aesthetically very appealing. But as with much modern art, the apparent surface simplicity is said to hide a deep inner meaning, which can only be properly discerned by the educated eye. In this book, a number of dedicated spin labellers try, successfully, to pass on the secrets of their art.

Probably the most useful part of the book for the experimentalist is the second half, in which applications of the technique are discussed. Four chap-

ters are concerned with membrane studies, a reflection of the fact that it is in the field of membranology that spin labelling has proved most fruitful. Particularly useful are discussions by J. Seelig, O. H. Griffith and P. Jost on the measurement of spin label order parameters, the overall conclusion being that simple measurements taken directly from the spectra usually give as good results as complex computations.

Two chapters deal with enzyme studies, but it is clear that here spin labelling has, in general, been rather less successful, in spite of the synthesis of a wide range of spin labelled compounds. The technique can be used to obtain distance information, but this is of course, information about the distance to an $>N-O$ group rather than to a part of the native enzyme. Most enzyme studies have, however, been of a much more qualitative nature, merely looking for changes in the spectrum following some perturbation of the system.

A further two very useful chapters provide details for the preparations of spin labelled compounds and discuss

instrumental aspects of obtaining electron spin resonance spectra. The first part of the book is the theoretical section containing chapters on general magnetic resonance theory by P. L. Nordio, on the theory of slow tumbling of spin labels by J. H. Freed and on the theory of biradicals by G. R. Luckhurst.

The only criticism that can be levelled at the book is that it does not meet its claim to be accessible to readers from medical and biological backgrounds. A quick survey of my medical colleagues showed that few knew what quantum mechanical operators or expectation values were, and such knowledge is assumed at the beginning of chapter two. It is a pity that the book does not include a non-mathematical discussion of the basis of electron spin resonance, since, in fact, the latter chapters show that detailed understanding of the theory is not necessary for its application to biological problems. Nevertheless, this is certain to become the spin labellers' benchside book, even if they do have to steal the library copy. **A. G. Lee**

Stimulus for ecologists

Ecological Animal Parasitology. By C. R. Kennedy. Pp. ix+163. (Blackwell Scientific: Oxford and London, 1975.) £3.50.

IN recent years a number of excellent books have been published on the general ecology and population biology of animal species with approaches ranging from the completely theoretical to practical treatments of field study problems. The ecology of two particular groups of organisms—protozoan and helminth parasites—has, however, received very little attention in general ecological texts, host-parasite relationships often being briefly dismissed by reference to the population equations of Lotka and Volterra.

Such treatment seems surprising, particularly when considered in relation to the economic importance of many host-parasite interactions. A careful examination of the parasitological literature reveals, however, that our knowledge of the ecology of the majority of parasitic organisms is very limited indeed. This unhappy state of affairs has undoubtedly arisen as a result of the complexity of many parasite life cycles, which may involve more than one species of host and many distinct parasite populations compounded by the difficulties in measurement and observation created by the intimacy of the relationship between host and parasite.

Clive Kennedy's new book is a welcome addition to the sparse literature on ecological aspects of parasitology. After a brief and perhaps rather superficial introductory chapter on the nature of parasitism, parasite population growth and host-parasite systems, the following four chapters are concerned with the concepts of host specificity and intra- and interspecific competition, the biology of host location and the dispersal and fecundity of parasites. Four of the remaining five chapters, constituting the major part of the book, deal in a fair amount of detail with the population biology of parasites within intermediate hosts, and poikilothermic and homiothermic definitive hosts. The contents of these chapters, reflect to a large extent the information available in the research literature, although they are somewhat biased towards the author's own interests in helminths and in particular parasites of fish. In the last chapter, perhaps the most stimulating of the book, the author attempts to come to terms with the dynamic aspects of the relationship between host and parasite populations. Some of the more quantitative attempts to model the population dynamics of host-parasite interactions are discussed, although it seems surprising that the pioneering and highly relevant work of V. A. Kostitzin 40 years ago is ignored.

This book will be of more general interest to students of parasitology than modern day ecologists since the author has not really achieved his stated objective of interpreting animal

parasitology in the light of current ecological ideas and concepts. For example, many rather important ecological topics such as the relationship between stability and complexity in host-parasite systems and the importance of time delays and genetic factors in population interactions are completely ignored. Furthermore, the author himself seems to be rather confused by certain ecological concepts, such as the role played by density-dependent factors in the regulation of population growth. He frequently refers to specific types of host-parasite interactions as "fundamentally unstable". The reader is first of all unaware of what the author means by stability and in addition is left to wonder how such interactions persist in the world today in the absence of regulatory mechanisms operating on population growth. In certain cases confusion is created by contradictory statements within the same paragraph. The author implying in one instant that mortality of parasites within poikilothermic hosts is a non-seasonal parameter and then going on to state that temperature influences the rate of loss of adult parasites from the host.

Many of the confusing discussions within this book are no doubt due to the lack of quantitative ecological information available in the parasitological literature. As an undergraduate text this book will be of value, and it may stimulate some of the more quantitatively minded ecologists to turn their attention to this fascinating field.

R. M. Anderson

Talking to computers

Conceptual Information Processing. (Fundamental Studies in Computer Science, Volume 3.) By R. C. Schank. Pp. viii+374. (North-Holland: Amsterdam and Oxford, 1975.) Dfl.75; \$31.25.

How can we get computers to understand natural language? One way is to restrict conversation to a very limited domain such as noughts-and-crosses or the world of children's blocks and then to try to devise programs that will go through most of the mental operations that human beings perform when they talk to each other about such topics. In spite of some promising developments, the disadvantages of this approach are obvious: change an aspect of the simulated world—introduce a baby to play with the blocks—and sooner or later the semantic machinery will be unable to cope. An alternative method is to restrict not the topic of discourse but what the programs do. Mechanical translation, the white hope that became a white elephant, was a lesson in the dangers of an extreme version of this approach. Nevertheless, Roger Schank has opted for a wide range of discourse at the cost of limited comprehension, and the present book describes his work in great detail down to three chapters by his students based on their PhD theses. They have contrived some ingenious programs that will generate a list of paraphrases of a sentence or draw plausible inferences from it, for example, given: "John said he killed himself", their system responds: "Dead people can't talk." The drawback is, of course, that the programs are not really demonstrating a full mastery of language. They could not describe a dead man even if they had eyes to see.

The choice at present seems to be either articulate conversations with an idiot savant who knows only how to pile up wooden blocks, or the inferential responses of a deaf, dumb and blind kid who cannot even play a pinball machine. Neither alternative promises to deliver us an intelligent talking computer by the year 2001. Perhaps we should treat computers like babies and provide them with senses and voices, the ability to learn how to represent the world and to reflect on their own processes, and then talk to them for a few years. **P. N. Johnson-Laird**

Manipulating plant growth

Environment and the Experimental Control of Plant Growth. (Experimental Botany: An International Series of Monographs.) By R. J. Downs and H. Hellmers. Pp. vii+145. (Academic: London and New York, November 1975.) £4.50; \$12.50.

MANIPULATING the environment is a technique used by experimental and applied botanists for many years in their search towards an understanding of plant growth, and the use of sophisticated controlled environment facilities has recently increased. The authors of this book have drawn on their considerable experience with such facilities to provide a monograph that will be welcomed by many researchers in the field. Their object was to collate information on environmental factors controlling plant growth and the methods of controlling these factors in turn, to enable biologists to understand what is required in the design and use of the facilities, in order to obtain acceptable degrees of control. In doing so, the authors have produced the first major book on the subject in over

ten years since L. T. Evans' *Environmental Control of Plant Growth*.

There are chapters covering temperature, light, the gaseous environment, and water and nutrients. Each begins with definitions, followed by methods of measurement and design of facilities, and examples of the influence that each factor has on the development and growth of plants. The technical information is presented with clarity. The physiological data is necessarily rather limited, but each chapter is concluded by a comprehensive reference list. The value of the book lies in its considerable amount of practical advice based on the authors' experiences, with emphasis on the problems that may be encountered. The book is concluded by chapters discussing the controlled environment in relation to the natural environment and the various types of facilities available. The authors are directors of the South Eastern Plant Environment Laboratories in North Carolina, and naturally stress the advantages of phytotrons since a number of facilities are available and visiting workers are encouraged there. Their book will, however, prove especially useful to those with more limited facilities and little experience. **S. M. Thomas**

Look—no maths

Electrons in Metals: An Introduction to Modern Topics. By C. M. Hurd. Pp. ix+331. (Wiley-Interscience: New York and London, December 1975.) £9.95; \$19.90.

DR HURD's book is an ambitious project—the descriptive treatment of the major properties of electrons in metals without the use of mathematics. The author sets out to lead beginners in the subject through the tangled web of quasiparticles, Fermi surfaces and Kondo effects to gain at least a qualitative overview of the theory of metals. To do this the author has developed what would, at first sight, seem to be a logical framework, in that he starts with an atomistic view so as to relate the various types of metals to the periodic table. He goes on, for what is the largest section of the book, to treat the electrons as a fluid with increasing complexity, from the single-particle model to Bose condensation and superconductivity, before introducing the atoms back into the problem for a general review of cohesion and electron transport in metals.

A very large part of metal physics is covered at a fairly elementary level but this book suffers, however, from a number of major faults, which are inherent in this format. If one is to

have a qualitative discussion of fairly sophisticated concepts it is necessary to provide a framework as simply and as early as possible. In the standard textbooks this would be achieved by either reference to the interplay between experiment and theory, which led to those concepts, or through a sufficiently simple model of a real metal that could be modified as one progressed in understanding. Neither of these has been followed by the present author and the simple single-particle model, on which the major part of the understanding of the electronic theory of metals can be based, is left until the final chapter. This means that many of the concepts, and indeed technical jargon, receive only the briefest mention on their initial appearance and the reader is constantly being referred forward to the later chapters. This would not necessarily be fateful, but coupled with the emphasis in large parts of the earlier parts of the book on the exception rather than the rule (as in, for instance, asymmetric scattering of electrons by impurities) must lead to terrible confusion for the beginner.

In general, although it does contain some very good non-mathematical descriptions of complex phenomena, this is not a book I would recommend for anyone without a good grounding in solid-state physics. **J. C. Inkson**

obituary

Dorothy Wrinch was born in Rosario, Argentina, on September 13, 1894 and died very recently on February 17, 1976 at Falmouth Hospital (Massachusetts). She was a brilliant and controversial figure who played a part in the beginnings of much of present research in molecular biology.

Her father was Hugh Edward Hart Wrinch, an engineer, and her mother, Ada Soutar. She was educated at Surbiton High School and won a scholarship to Girton College, Cambridge, where she read mathematics—she was a Wrangler in 1916—and moral sciences. She was appointed to a lectureship in pure mathematics at University College, London, in 1918 and became the first woman ever to receive a D.Sc. from Oxford (1929). In the early 1920s she was one of a brilliant circle of mathematicians and physicists; among her friends were Dora Black and Bertrand Russell, whom she introduced to each other. In 1922, she married John William Nicholson, FRS, then a fellow of Balliol College, Oxford, and well known for his work on atomic structure. The marriage became more and more unhappy as his mental state deteriorated; it was finally dissolved in 1937 leaving Dorothy with one daughter, Pamela. From 1922 until 1939 Dorothy Wrinch lived mainly in Oxford, holding various research fellowships and a lectureship and tutorship in mathematics at Lady Margaret Hall and Somerville College. In 1939 she took Pamela to America where she met and married, very happily, Otto Glaser, Professor of Biology at Amherst, and spent the rest of her life in the USA, holding appointments in mathematics and physics at Amherst, Smith and Mount Holyoke until she retired to Woods Hole, which she greatly loved.

Until 1932, Dorothy Wrinch's research work was all in the field of pure mathematics, particularly classical analysis. In 1930, she turned towards

biology and set herself seriously to study the facts of elementary biology and chemistry by attending courses in Vienna and Paris during her sabbatical terms. She then began to seek for mathematical solutions to the problems she found, particularly of the structure of chromosomes, including nucleic acids and proteins. It was the properties of protein films that first suggested to her that peptide chains should be polymerised into sheets, by links between CO and NH groups to which she gave the name 'cyclol'. Folding the sheets then gave her a beautiful series of closed octahedra and other solid figures, built of definite numbers, n , of amino acid residues; one series has the general formula $72n^2$. These provided an attractive explanation of many recent observations on proteins, for example, the limited number of sizes of protein molecules found by Svedberg's early ultracentrifuge measurements. When Bergmann and Niemann in 1937 deduced from chemical analysis that egg albumin had 288 residues in the molecule, the exact number required for the second member of the above series, 72×2^2 , Dorothy Wrinch felt her hypothesis had been strikingly verified. The coincidence was extraordinary. Her infectious enthusiasm carried many with her: almost all the participants of the first Cold Spring Harbour Symposium on proteins in 1938 and, most notably, Irving Langmuir. Her explanation of the first X-ray evidence on the structure of insulin positively encouraged others to take up the study of protein crystal structures, W. L. Bragg and David Harker among them. (Bragg used her version of the insulin Patterson maps as one of the illustrations in his last book.)

Gradually and relentlessly, after the war, chemical and crystallographic work proved that protein molecules did not have the cyclol structure. Yet many of Dorothy Wrinch's geometrical

instincts and deductions were, in general terms, correct. The structures of protein molecules are *not* based on simple parallel arrays of peptide chains, yet the packing of molecules within the insulin unit cell is octahedral in character, there are patterns of close packed units over closed surfaces in many spherical viruses, even the 'cyclol' link itself sometimes exists, for example in the ergot alkaloids. This pleased her—she found it very hard to give up the cyclol theory as a whole. She maintained to the end of her life a strong interest in crystallography and in biology; the book she wrote at the request of her crystallographer friends on *Fourier Transforms and Structure Factors* is still very useful. And in old age she came again to Oxford to talk over the structure of insulin.

I like to think of her as she was when first I knew her, gay, enthusiastic and adventurous, courageous in the face of much misfortune, and very kind.

Dorothy Crowfoot Hodgkin

Dorothy Wrinch's early work was in pure mathematics, with occasional excursions into philosophy. She helped with the proofs of the 1920 edition of Whittaker and Watson's *Modern Analysis*. She wrote papers on mathematical logic in the tradition of Whitehead and Russell and, in the early 1920s, a number of papers on hypergeometric functions and problems in classical mechanics. She and I published four joint papers in the *Philosophical Magazine* on the theory of scientific method. I should like to put on record my appreciation of the substantial contribution she made to this work, which is the basis of all my later work on scientific inference.

We also had a joint paper on the seismic waves from the Oppau explosion of 1922, which was the first co-ordination of seismic data with the structure of the upper layers inferred from geological evidence by Suess.

Harold Jeffreys

Reports and publications

United States Department of the Interior: Geological Survey. Bulletin 1395-C: Stratigraphic Nomenclature of the Thirtynine Mile Volcanic Field, Central Colorado. By Rudy C. Epis and Charles E. Chapin. Pp. iii+23. (Washington, DC: Government Printing Office, 1974.) 50 cents. [191]

Airports and the Environment. Pp. 290. (Paris: OECD; London: HMSO, 1975.) 40 francs; £4.40; \$10. [191]

Twenty-third Annual Report of the Australian Atomic Energy Commission, 1974/1975. Pp. 132. (Coogee, NSW: Australian Atomic Energy Commission, 1975.) [191]

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